Taxa vs Traits: analysing stream communities in South Australian dry environments

A temporary stream in Polipotamos, Greece, Europe (Photo by Stam Zogaris)

Paul Kingsley Botwe (MPhil, BSc)

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Statements by the author

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Paul Kingsley Botwe

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Statement of co-authorship

The following people and institutions contributed to the work undertaken as part of this thesis:

Paul K. Botwe (University of Tasmania) was the primary investigator and lead author of the publishable manuscripts, and led all aspects of each study including the development of ideas, study design, analysis of data, interpretation of results and the writing of all the manuscripts.

Scott Carver (University of Tasmania) was the primary supervisor of the research and guided the development of ideas, study design, analysis of data, interpretation of results, and producing publishable manuscripts (1), (2), (3) and (4), as well as guiding and editing the other chapters of this thesis.

Leon A. Barmuta (University of Tasmania) assisted with guidance and supervision of the research relating to the development of ideas, study design, analysis of data, interpretation of results, and producing publishable manuscripts (1), (2), (3) and (4), as well as guiding and editing the other chapters of this thesis.

Regina Magierowski (University of Tasmania) assisted with guidance and supervision of the research relating to the development of ideas, study design, analysis of data, interpretation of results, and producing publishable manuscripts (1), (2), (3) and (4), as well as guiding and editing the other chapters of this thesis.

Peter Goonan (Environment Protection Authority, South Australia) and Paul McEvoy (Department of Environment, Water and Natural Resources, Adelaide, South Australia) supervised and collected the data sets on which this thesis was based as part of State- and Federally-funded programs. They also helped with the development of idea, and producing publishable manuscripts (1), (2), (3) and (4).

Chris Madden (Freshwater Macroinvertebrates, 28 Kingswood Crescent, Lockleys, Adelaide, South Australia, 5032) assisted with guidance of the research relating to the development of ideas, data collection, and producing publishable manuscripts (3) and (4).

We the undersigned agree to the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed:

Dr. Scott Carver
Primary Supervisor
School of Biological Sciences
University of Tasmania
Date: 23rd October, 2017

Signed:

Prof. Anthony Koutoulis
Head of School
School of Biological Sciences
University of Tasmania
Date: 24th October, 2017

Signed:

Ass/Prof. Leon Barmuta
Co-supervisor
School of Biological Sciences
University of Tasmania, Australia
Date: 24th October, 2017

Signed:

Dr. Regina Magierowski
Co-supervisor
School of Biological Sciences
University of Tasmania, Australia
Date: 23rd October, 2017

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Abstract

The availability of water is critical to ecosystem functioning and human health, but it is impacted by anthropogenic activities, such as those associated with intensification of surrounding land use. These can result in a range of aquatic impacts, including: changes to water quality, alteration of aquatic communities and modification of flow regimes. Consequently, stream ecosystems are monitored or assessed using a range of techniques to inform management strategies that attempt to minimise deleterious impacts and maintain the ecological integrity of stream ecosystems.

This thesis evaluates two important approaches to assessing biological conditions in stream ecosystems: taxonomic and trait-based approaches. Many studies have assessed stream condition using taxonomic characteristics of macroinvertebrates (i.e., their identities and abundance). While valuable, limitations of this approach are that taxonomic studies are restricted in their extendibility across studies, owing to biogeographic variation in species identities across different regions, and low proportion of variance explained when relating taxonomic composition to environmental factors. Further, taxonomic approaches often do not improve our understanding of the causal mechanisms by which impacts occur. In response to these limitations, functional traits of macroinvertebrates have been proposed as alternative measures of the biological condition of streams. This proposal is based on the potential for trait quantification to overcome biogeographic effects, potentially explain greater variation in aquatic community composition, and improve our understanding of the casual mechanisms by which environmental disturbances affect ecological communities.

Using 13 years of data sampled biannually across multiple sites in South Australia, I first undertook a traditional, taxonomic-based study. I related land-use, geographic and environmental variables to community composition in order to determine the most influential physical variables structuring these communities (Chapter 2); in addition to the known importance of flow. I found that physical variables explained 23.7% and 27.3% of the variation in taxonomic space in autumn and spring respectively, with salinity being the most strongly associated variable to community structure in both seasons. Geographic location variables (latitude and longitude) were also predictive of community structure in both seasons, suggesting a strong biogeographic effect on communities. Subsequently, I focussed on salinity and flow as predictors of macroinvertebrate community structure.

Secondly, I systematically reviewed the effects of flow and salinity on macroinvertebrate traits (Chapter 3) to better understand the temporal and geographic extent of previous studies, the methodological and analytical approaches used to relate flow and salinity to macroinvertebrate traits, and examine the extent to which there have been consistent outcomes across studies. I found that studies were accumulating at a slow and steady rate (zero to two papers published per year for flow-traits and salinity-traits over the last decade). The geographic coverage of studies was strongly biased towards North America and Western Europe. There were broad trends in which traits appear to be associated with flow and salinity, but there were also inconsistencies in some trait responses to effects of these stressors. These inconsistencies may be due to differences in analytical and methodological approaches among studies. I did not find any studies that examined interactions between effects of flow and salinity on macroinvertebrate traits, even though low flows and high salinities often co-occur and may interact, especially in temporary streams. Thus, I hypothesized that interactions between flow and salinity may underlie some inconsistencies in results across the trait literature.

As a result, I investigated the simultaneous effects of flow and salinity on macroinvertebrate traits to examine the extent to which their individual and interactive effects were important (Chapter 4). I found that traits showing inconsistent behaviour in the literature were indeed associated with interactions between flow and salinity, which appeared to be driven by the differential responses of taxa with the same trait. Further, flow and salinity variables explained more of the variation in trait space (27.3% in autumn, 36.7% in spring) than taxonomic space (20.9% in autumn, 27.7% in spring), but there was still strong site-specific effects which suggested that biogeographic effects remain, even for trait composition.

To compare traits with taxonomic approaches in more detail independent of biogeographic effects, I analysed data separately within four sites with the most complete flow and environmental records from my South Australian dataset (Chapter 5). I evaluated two perennial and two intermittent flow regimes. I predicted that trait composition would be more similar between seasons than taxa, because there is more turnover with taxa over time than with traits. I also predicted that for both taxonomic and trait composition, intermittent streams would be less similar between seasons than perennial streams because intermittent streams are more likely to lose species by chance during dry periods. These expectations were generally supported by the data. Moreover, trait structure was better explained by flow and salinity than taxonomic structure, although the differences were heterogeneous, ranging from 2% to 40% of additional variation explained. Differences in variation explained were

associated with the number of taxa and their pattern of dominance sharing the same trait, and not associated with stream flow regime.

This thesis compared and contrasted taxonomic and trait based approaches to evaluating stream ecosystems, with a focus on the effects of flow and salinity. I showed that flow and salinity were important predictors of aquatic macroinvertebrate taxonomic and trait composition in South Australia, and that the interactive effect of these variables was also important, explaining some inconsistences among studies in the literature. Consistent with theory, greater variation was predicted for trait-space than taxonomic. However, in contrast to theory, I did not find that trait-based approaches overcame biogeographic effects. Phylogenetic relatedness among traits and differences in methodological approaches used to relate traits to environmental conditions pose challenges in trait-based analysis, and need to be addressed in future studies. To foster the operative use of species traits for stream bioassessment, there is the need for comprehensive regional or national trait databases resolved at species level, and standardized in terms of trait classification and nomenclature.

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Chapter 1

General introduction

1.1 Natural and anthropogenic disturbances as drivers of change in freshwater ecosystems

Freshwater ecosystems cover approximately 0.8% of the earth's surface and contains less than 0.01% of the world's freshwater (Dudgeon et al., 2006, Wetzel, 1975). Of these scarce freshwater systems, rivers and streams are arguably the most relied upon by humans (Malmqvist and Rundle, 2002). As a result, streams and rivers have become the most altered and threatened ecosystems in the world (Malmqvist and Rundle, 2002), with the biota exposed to a range of natural (Poff et al., 1997) and anthropogenic disturbances (Botwe et al., 2015) of varying strength, frequency, duration, predictability, and at varying spatial scales. Such disturbances which arise from either natural (e.g. natural flow variability) or anthropogenic (e.g. land-use) sources influence stream biota, disrupt ecological processes, and redistribute resources (Lake et al., 2000).

As a result, streams and rivers are assessed using a range of methods, including diversity indices, biotic indices, multi-metric approaches, among others (Li et al., 2010). Ensuring that the appropriate techniques are used to assess river conditions is important because human use of streams and rivers, and their surrounding catchment is only expected to increase. In the light of this expanding human footprint and intensification of land-use, it is therefore imperative that anthropogenic disturbance of streams and rivers is monitored and assessed appropriately. This will foster the development of management strategies that minimise negative effects and maintain the ecological integrity of these freshwater ecosystems. This thesis assesses the relative performance of taxonomic-based and trait-based approaches for analysing conditions in stream ecosystems. This was achieved using a large dataset derived as part of the Australian Rivers Assessment System (AusRivAS) (Davies, 2000) of which South Australia has been part since 1994.

1.2 Taxonomic approach

Traditionally, 'stream condition' is assessed using the relative abundances of macroinvertebrates (i.e. a taxonomic-based approach) (Palmer et al., 2015). This is practical

because the occurrence, abundance and distribution of macroinvertebrates are affected by environmental variability (Moreno et al., 2010), arising from natural and anthropogenic sources. Thus, by understanding how macroinvertebrate taxa respond to environmental factors, managers can develop guidelines for use of rivers and streams. In view of this, a number of biotic indices that include both quantitative species diversity measures (e.g. species richness, evenness and diversity) and qualitative data on the sensitivity of individual taxa [e.g. EPT (percent of Ephemeroptera, Plecoptera and Trichoptera)] to environmental changes have been developed (Gonçalves and Menezes, 2011).

However, taxonomic approaches to biomonitoring have limitations. First, taxonomic studies are restricted in their extendibility across studies owing to biogeographic variation in species identities across different regions (Statzner and Bêche, 2010) (Table 1). Second, the use of taxonomic identities does not improve our understanding of the causal mechanisms by which impacts occur (McGill et al., 2006). Lastly, many studies associating macroinvertebrate composition with environmental factors have low explained variance (5 - 30%) (Harris and Heathwaite, 2012). These limitations have led to exploration of alternative approaches to biomonitoring, and one approach, which has gained recent momentum is the use of macroinvertebrate traits.

1.3 Traits as an alternative to taxonomic-based analysis

A biological trait is a characteristic of a species that is used to define a biological feature of an organism or its direct relationship with the environment (Poff et al., 2006). The habitat templet model of Southwood (1977, 1988) forms the basis of the concept of a correspondence between species' traits and prevailing environmental conditions. The theory is based on the assumption that the habitat provides the templet on which evolution forges characteristic life-history strategies (Southwood, 1977 and 1988). This theory was later developed into the river habitat templet concept by Townsend and Hildrew (1994), in relation to stream fauna, and was tested by evaluating the relationships between community trait composition and major environmental gradients (Dolédec et al., 1999, Townsend et al., 1997). Their findings revealed that the use of biological traits more reliably indicated human impact than the conventional taxonomic approach. This then stimulated interest in the application of trait-based approaches in biomonitoring. Since then, trait-based approaches have been extended to include different organisms group including terrestrial plants (Bonan et al., 2012, Hoffmann et al., 2012) and birds (Leveau, 2013, Sekercioglu, 2012). The concept of how traits is linked

to ecosystem functioning is based on identifying specific biological traits that have the strongest influences on particular ecosystem processes and contribute most to functional diversity (Tilman, 2001). This may provide a crucial link in the development of a broader framework for understanding how species composition and diversity affect ecosystem functioning and by extension, the delivery of ecosystem services (Astegiano et al., 2015). For example, body size and growth rates are clearly traits that can both influence the capacity of an organism to respond to the environment (with stress often favouring small-sized, fast growing taxa), and also their effects on processing of resources and community-level productivity (Truchy et al., 2015).

First, using traits may be able to overcome the difficulty of extrapolating between geographically disparate systems that might differ taxonomically (Statzner et al., 2004). This is because different geographic regions are often characterised by different taxonomic compositions, but are likely to contain similar complements of biological traits (Johnson, 2005). Thus traits may potentially increase the spatial applicability or extensibility of studies (Usseglio-Polatera et al., 2000a) which is essential if assessment tools are to be comparable among states or countries that are subjected to the same legislation, in terms of land and water use (OJEC, 2000) (Table 1).

Table 1. Summary of advantages of traits over taxa

Trait-based	**Taxonomic-based**
May overcome biogeographic effects	Does not overcome biogeographic effects
May improve understanding of causal mechanisms	Does not improve understanding of causal mechanisms
May explain more variation attributed to environmental factors	Explains less variation attributed to environmental factors

Second, using traits in biomonitoring may present the possibility of developing a mechanistic framework linking the occurrence and distribution of traits in ecological communities to environmental stressors. This may improve the sensitivity of bioassessment (Culp et al., 2011, Rubach et al., 2011) and provide insight regarding the sources of impairment.

Third, incorporating traits into biomonitoring programmes will not require the formulation of new biomonitoring framework; rather, it can be integrated into current assessment programmes without additional data collection or sampling effort. This is feasible because contemporary biomonitoring programmes, using standard analytical and sampling protocols, still gather the basic site-by-species composition matrices needed to link community taxonomic data to trait databases, and existing databases may provide the needed trait information (Culp et al., 2011).

The improved ecological relevance gained by using traits may be applied to a number of organisms including invasive species to understand their success and persistence. For example, studies have described traits responsible for successful plant invasion (Küster et al., 2008), habitat loss (Hockey and Curtis, 2009) and bird introductions (Vall-llosera and Sol, 2009). Further, the use of traits may provide useful information in the assessment of emerging issues such as the identification of novel traits in genetically modified organisms that pose a risk to the environment and other species (Snow et al., 2005).

However, it is worth noting that the outcome of trait-based community patterns may be influenced by the taxonomic resolution of the taxa characterised by traits (family, genus, species or mixed), the number and type of traits being considered, trait data conversion (quality) and how trait-environment relationships are quantified (Heino et al., 2013). For instance, studies that are unable to identify the more cryptic taxonomic groups (e.g. chironomids and oligochaetes) to lower taxonomic levels either use coarse taxonomy (e.g. Cid et al., 2016) or exclude them from the analysis (e.g. Gallardo et al., 2009). However, omitting such taxa could restrict the suite of trait categories available for identifying the type and level of environmental impact (Culp et al., 2011), while assigning traits at coarse taxonomic resolution may obscure important responses as demonstrated by Serra et al. (2016). These challenges are a major impediment to using trait-based approaches.

Many studies have explicitly assessed stream conditions in relation to environmental factors across catchments scales (Bêche and Statzner, 2009) using taxonomic-based methods, with fewer focussing on macroinvertebrate traits (Velghe and Gregory-Eaves, 2013). To improve our understanding of the effects of landscape modification on aquatic ecosystems, comparative analyses of taxonomic and trait-based approaches are needed. For this thesis, I compared and contrasted conventional taxonomic approaches with trait-based approaches to examine which approach provides a better measure of stream ecological state.

1.3.1 Traits and methodological approaches

Generally, species-trait databases have been compiled using a fuzzy coding approach of Chevenet et al. (1994) that assigns affinity of species to trait categories that have been defined from existing biological knowledge. The degree of association or affinity can take values from "0" to "5" (depending on the number of trait categories within a trait group), with "0" defined as no association or no affinity, and "5" as strong association or high affinity (Astor et al., 2014, Usseglio-Polatera et al., 2000b). The affinity scores were treated as relative distributions (among the set of categories for each trait) and standardized to sum 1, to give the same weight to each taxon and to each trait in further analysis (Chevenet et al., 1994, Mondy and Usseglio-Polatera, 2014). This procedure is known as fuzzy coding, and it is a simple way to "translate" the available knowledge about traits of organisms into numerical values (Chevenet et al., 1994).

The identification of a set of traits, which respond to changes in environmental conditions, is key for functional ecology (Bernhardt-Römermann et al., 2008). However, the methodological challenge associated to this goal relies on the analysis of the information contained in three tables: a table R ($n \times m$) with the measurement of m environmental variables in n samples (e.g. site), table L ($n \times p$) with the abundances (or occurrences) of the p species within n samples, and a third $p \times s$ table Q describing s traits for p species (Dolédec et al., 1996). Several approaches have been developed to examine the link among these tables. For instance, some authors (e.g. Mabry et al., 2000, Pakeman and Marriott, 2010) combined the Q and L to construct a sample × trait table that contains for each sample the (weighted by species abundances) averages of numerical traits over all species present or the (weighted) frequencies of categorical traits. The link between the sample × trait and the R matrices can then be investigated using a two-table ordination method. Other authors use a two-step approach (indirect functional analysis) where tables R and L are first analysed and then the results are interpreted using species trait data (Thuiller et al., 2004), while a few authors have used the fourth-corner approach: a direct functional analysis where species, environment, and species-trait data are analysed simultaneously (Dray et al., 2014, Lehsten et al., 2009).

Further, based on the observed effects of species traits on ecosystem functioning, a number of indices have also been proposed to measure functional composition and diversity

of a given community (Lepš et al., 2006). These indices involve *a priori* classification of species into functional groups, the sum (Walker et al., 1999) or average (Schmera et al., 2009) of functional distances between species pairs in multivariate functional trait space, or dendogram-based measures (Petchey and Gaston, 2002). Thus, a number of indices such as "community-weighted mean trait values" [CWMs] (Garnier et al., 2004) and Rao coefficient (Pavoine and Dolédec, 2005) have been widely adopted in ecological research for summarizing different aspects of community trait composition.

1.4 Australian Rivers Assessment System (AusRivAS)

In 1993, the commonwealth Government of Australia established the National River Health Program (NRHP), to develop a national coordinated approach to river health monitoring. One of the main components of the NRHP is the Australia-wide Assessment of River Health (AWARH), which consists of a nation-wide evaluation of the ecological condition of rivers using a set of rapid, standardised and integrated methods, known as the Australian River Assessment System (AusRivAS), as well as a series of toolbox projects aimed at developing and refining the set of existing assessment techniques (Davies, 2000, Simpson and Norris, 2000) [https://ausrivas.ewater.org.au/index.php/resources2/category/19-manuals, accessed July 2017]. The NRHP also involves the development of predictive models to assess the health of rivers and streams using macroinvertebrates sampled from a wide range of riverine habitats (Davies, 2000, Simpson and Norris, 2000). An important component of this is for macroinvertebrates as well as a range of physical and chemical data to be collected from a number of near pristine reference sites, selected to be either near-pristine or as undisturbed by humans as possible, given the regional context of the sampling. The number and type of macroinvertebrates collected from a disturbed ('test') site were then compared with those expected in reference sites, providing a measure of the biological impairment at the test site. This formed the basis of the bioassessment.

1.4.1 Field sampling procedures in AusRivAS

Macroinvertebrates were sampled separately from two habitats, riffle and edge (pool), where present, using standard 250 μm mesh dip net. Riffle samples were collected by the operator facing downstream, placing the net directly on the substratum in front of the feet and vigorously disturbing the substratum by kicking and twisting the feet to a depth of approximately 10 cm, and slowly moving upstream. This action dislodged organisms, which

were then swept into the net. Macroinvertebrates collected in pool habitats involved sampling representative 5 m^2 area of the habitat within each 100 m study site. This habitat was also sampled by vigorously kicking the substrate and sweeping the net over a total bank length of 10 m, using sequential short sweeping movements at right angles to the bank and, sweeping under overhanging or emergent vegetation (Davies, 2000).

After sampling a habitat, small portions of the contents of the dip net were emptied into a sorting tray and macroinvertebrates were live-picked on site with fine forceps into small vial of 100% alcohol, labeled with site name, location code, date, habitat type and collector's initials. The live-sort was done until the entire sample had been sifted through. This live-sort strategy involved a minimum of 30 minutes pick whereby the first five minutes were spent picking abundant taxa, then the rest of the time was directed at finding the less common and inconspicuous taxa. If after 30 minutes, the number of individuals collected were less than 200, then picking continued for a further 10 minutes, and if a new taxon was encountered within that time period, a further 10 minutes was added to the picking time. However, the total picking time would not exceed 60 minutes (Davies, 2000). The preserved macroinvertebrate samples were then transferred to the laboratory for counting and identification, given available taxonomic keys. The residues of at least 10% of all samples were randomly preserved for internal and external quality control and quality assurance, in order to check operator-picking efficiency. In addition to sampling macroinvertebrates, a suite of environmental variables including water quality and habitat characteristics were also recorded for each sampling site.

1.5 The data set

To achieve the overall aim for my thesis, I utilised a large dataset derived as part of the Australian Rivers Assessment System (AusRivAS) (Davies, 2000) of which South Australia has been part since 1994. The database includes a substantial, standardised record of benthic macroinvertebrates and a large number of environmental variables. These collections were subjected to QA/QC protocols (https://ausrivas.ewater.org.au/index.php/resources2/category/19-manuals, accessed July 2017). Sampling was conducted annually in two seasons (autumn and spring), to avoid dried-out summer periods and low macroinvertebrate activity during winter. I used data collected for 13 years from 1994 to 2007, excluding 1996, when there was insufficient funding for

sampling. Collected macroinvertebrates were laboratory sorted, thus ensuring observer bias was minimised when counting individuals compared to alternative live-pick approaches included in the AusRivAS protocols, and it also provided an accurate estimation of the abundance of cryptic taxa. Further, taxa were identified to the lowest taxonomic level, given available keys, life-history stage and condition. This was most often to genus or species level (including the oligochaetes and chironomids), with voucher specimens of all taxa retained as a reference collection at the South Australia Museum and Australian Water Quality Centre (AWQC).

I used 13 sites sampled consistently across multiple catchments (Fig. 1). There are well-defined gradients of flow (rain shadow effect from West to East) and salinity (increases from Adelaide towards the North of South Australia) across the study sites. Flows in these streams are largely driven by ground water, which has accumulated marine-derived salts over long time-scales (Herczeg et al., 2001). In places, clearing of native vegetation and irrigation for agriculture further exacerbates salinity. There are no secondary sources of salinization such as from mining, coastal inundation, industrial discharge or road de-icing, and electrical conductivity is dominated by Na^+ (median: 72 % of cations) and Cl^- (median: 98% of anions), with similar ionic compositions across this region. I used the South Australian dataset set because it has well-resolved taxonomy (the best in the country), spans multiple sites and years, and has important environmental variables characterised.

1.6 Study region

The study was conducted in South Australia with my sites distributed throughout Kangaroo Island (1 site from Rocky River), Yorke and Fleurieu Peninsulas (2 sites including Hill River and Kanyaka Creek), the Mount Lofty Ranges (MLR) [Western MLR = 7 sites including Hindmarsh, Torrens, North Para, Myponga and Light rivers, First and Scott creeks] and the lower Mid-North of South Australia (3 sites including Finniss, Marne and Bremer rivers) [Fig. S1 in Supporting Information]. Flows in these streams are largely driven by ground water which has accumulated marine-derived salts over long time-scales (Herczeg et al., 2001). In places, salinity is further exacerbated by irrigation and clearing of native vegetation for agriculture. There are no secondary sources of salinization such as from mining, coastal inundation, industrial discharge or road de-icing, and electrical conductivity is dominated by Na^+ (median: 72 % of cations) and Cl^- (median: 98% of anions), with similar ionic

compositions across this region (Supporting Information Table S1). I therefore referred to conductivity as salinity in my study.

I studied 13 sites: six perennial (Finniss, Myponga, Rocky and Hindmarsh Rivers, Scott and First Creeks) and seven intermittent (Hill, Light, Torrens, Bremer, North Para and Marne Rivers, Kanyaka Creek). Finniss River (FR) has a catchment area of 187.2 km^2. It is a large stream in the southern Mount Lofty Ranges that rises east of Yundi and flows in an easterly direction where it eventually discharges into the Lower Murray, northeast of Goolwa (Armstrong et al., 2003). Scott Creek (SC) has a catchment area of 26.7 km^2 and is a sub-catchment of the Onkaparinga River to the south east of Adelaide. Grazing comprises the major land use in this catchment with native vegetation existing on the steeper slopes of the catchment (Deane, 2012). The climate of both catchments are typically temperate and rainfall tends to occur in winter and spring with the bulk of the rainfall in the winter months (Harrington, 2004).

Kanyaka Creek has a catchment area of 177.2 km^2 and rises near Hawker and discharges into Willochra Creek, about 5 km north from Simmonston. The major land uses in this catchment, upstream from the site sampled, are grazing modified pastures (84%) and grazing natural vegetation (15%) (Deane, 2012). Hill River has a catchment area of 245.8 km^2 and occurs approximately 3 km east of Penwortham and flows north to join Yakilo Creek to form the Broughton River (Deane, 2012) [Supporting Fig. S1]. Both streams are highly intermittent (usually 100-200 zero-flow days per year) (Kennard et al., 2010).

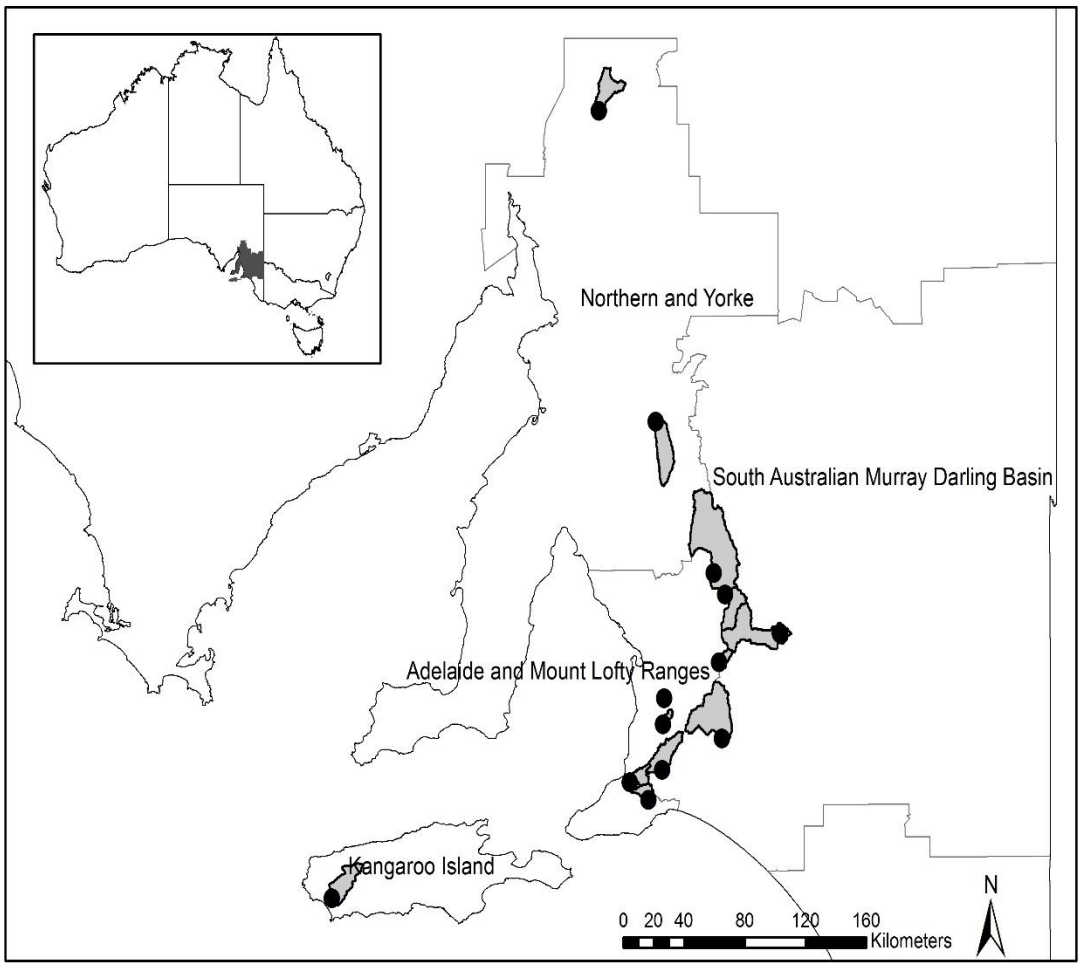

Fig. 1. Map of South Australia, showing the distribution of the sampling sites used for the study. Circles represent study sites; catchment boundaries upstream of study sites denoted by thick black lines; coastline and state borders in grey lines; thin black lines represent boundaries to NRM regions.

1.7 Thesis aims

The overall aim of this thesis is to assess the relative performance of taxonomic-based and trait-based approaches used in analysing conditions in stream ecosystems, in order to determine whether models perform better when examining traits over taxa. The focus of each chapter was partially informed by the outcomes of the preceding chapters. Thus, I have four specific aims, which are as follows:

1) To investigate temporal patterns and environmental correlates of macroinvertebrate communities in temporary streams. This enabled me determine the most influential

physical variable driving the community composition, in addition to known importance of flow (chapter 2)

2) To systematically review the trait-based literature of lotic macroinvertebrate responses to effects of flow and salinity. From this, I was able to examine if there are consistent or inconsistent outcomes across studies regarding traits response to effects of flow and salinity (chapter 3)
3) To investigate the effects of salinity and flow interactions on macroinvertebrate traits in temporary streams. This enabled me to understand whether interactions underlie inconsistencies in the literature about the responses of some traits to flow and salinity (chapter 4)
4) To analyse within-site data sets in order to move away from the biogeographic signal that affected taxonomic and trait composition. From this, I was able to ascertain whether models better predicted traits than taxa in response to abiotic factors (chapter 5)

1.8 Thesis structure

Chapters' two to five of this thesis are written as individual research manuscripts, which have been published, submitted or intended for submission for publication. Thus, some repetition of the methods or derived material occurs across chapters.

Chapter 2

Temporal patterns and environmental correlates of macroinvertebrate communities in temporary streams

Photo by Amy Marcarelli

This chapter is published as:

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2.1 Abstract

Temporary streams are characterised by short periods of seasonal or annual stream flow after which streams contract into waterholes or pools of varying hydrological connectivity and permanence. Although these streams are widespread globally, temporal variability of their ecology is understudied, and understanding the processes that structure community composition in these systems is vital for predicting and managing the consequences of anthropogenic impacts. We used multivariate and univariate approaches to investigate temporal variability in macroinvertebrate compositional data from 13 years of sampling across multiple sites from autumn and spring, in South Australia, the driest state in the driest inhabited continent in the world. We examined the potential of land-use, geographic and environmental variables to predict the temporal variability in macroinvertebrate assemblages, and also identified indicator taxa, that is, those highly correlated with the most significantly associated physical variables. Temporal trajectories of macroinvertebrate communities varied within site in both seasons and across years. A combination of land-use, geographic and environmental variables accounted for 24% of the variation in community structure in autumn and 27% in spring. In autumn, community composition among sites were more closely clustered together relative to spring suggesting that communities were more similar in autumn than in spring. In both seasons, community structure was most strongly correlated with conductivity and latitude, and community structure was more associated with cover by agriculture than urban land-use. Maintaining temporary streams will require improved catchment management aimed at sustaining seasonal flows and critical refuge habitats, while also limiting the damaging effects from increased agriculture and urban developments.

2.2 Introduction

Temporary streams, characterised by the repeated onset and cessation of flow are widespread globally and common in agricultural and urban landscapes (Eng et al., 2015, Leigh et al., 2015, Tooth, 2000). Macroinvertebrate communities in these streams are thought to be largely driven by flow variability, and this temporal variability is an important, but understudied, aspect of their ecology. Although our understanding of spatio-temporal variation in patterns of macroinvertebrate community structure in permanent streams has greatly improved (Amoros and Bornette, 2002), few studies have examined temporal variability of macroinvertebrate communities in temporary streams (Leigh and Sheldon, 2009).

Temporary streams present challenges for assessing environmental condition and impacts owing to their inherent variability. For example, stream condition is often assessed with predictive models based on macroinvertebrate occurrence at reference sites that are only minimally affected by human disturbance. This is the case in Australia where the observed/expected predictive models developed as part of the Australian River Assessment System [AUSRIVAS] (Norris et al., 2007, Simpson and Norris, 2000) are based on the assumption that macroinvertebrate assemblages are relatively spatio-temporally consistent in the absence of anthropogenic perturbation, and that sampling sites are suitably similar (or undisturbed enough, in the case of reference sites) to allow robust comparison (Blanchette and Pearson, 2012). However, suitable intermittent reference sites have not been used because these ideal reference conditions are difficult to define in highly seasonal dry-land streams, and demonstrating that changes in macroinvertebrate assemblages are caused by anthropogenic disturbance is difficult when the natural variability of assemblages in such systems is unknown (Chessman et al., 2010). Furthermore, Larned et al. (2010) argued that water managers usually mis-manage temporary streams by applying perennial stream management principles, thus leading to potentially erroneous decisions about best management practices.

Despite global concern over current and future land-use impacts, little attention has been given to the effects of land-use on temporary streams (Rose et al., 2008), primarily because of the episodic nature of these streams (Boulton, 2003). Reviews by Johnson and Host (2010), Steel et al. (2010) and Allan (2004) have highlighted the need for an improved understanding of the mechanisms by which land-use and related environmental variables alter stream biota and habitats.

In this study, we explored relationships of macroinvertebrate community composition to land-use, geographic and environmental variables in the Mount Lofty Ranges and Kangaroo Island; two warm temperate regions in South Australia with a largely Mediterranean climate. Temporary streams are abundant in South Australia (Laut et al., 1977) and include a spectrum of annual flow-cessation regimes in a state where land-use varies in its intensity. Our study spanned multiple sites sampled in two seasons for 13 years, with sites varying in terms of the proportion of land-uses in their upstream catchments. Some catchments in our study are typically temperate and rainfall tends to occur in winter and spring with the bulk of the rainfall in the winter months (Harrington, 2004). This study will

enable us determine the most influential physical variable driving the community composition in addition to known importance of flow. In this study, we aimed to: (i) explore the temporal variability and trajectories of macroinvertebrate composition; (ii) examine the potential of land-use, geographic and environmental variables to predict macroinvertebrate assemblages and; (iii) identify indicator taxa that are correlated with gradients of specific land-uses, environmental and geographic variables.

2.3 Materials and methods

2.3.1 Ethics Statement

This research did not involve vertebrates or cephalopods and therefore was not required to be approved by the Animal Ethics Committee of the National Parks and Wildlife Division of the South Australian Department of Environment and Heritage, which complies with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th Edition, 2013), the Prevention of Cruelty to Animals Act 1985 (South Australia), and the Australian Code for the Responsible Conduct of Research (2007). All sites sampled (see Table S3) were on private state or crown lands and permits were not required. The Department of Environment, Water and Natural Resources (South Australia) who manage crown lands confirmed that no permits were required for access to crown land. Land title details can be found on the South Australian Integrated Land Information System ('SAILIS' www.sa.gov.au/topics/housing-property-and-land/land-services-industry/sailis). Most taxa sampled in this research were identified to species and no taxa were listed as endangered or protected in Australian state or federal legislation.

2.3.2 Study area and macroinvertebrates sampling

Our study sites were in four of the eight Natural Resource Management (NRM) regions in South Australia. Sites include the Adelaide and Mount Lofty NRM region (Western MLR = 7 sites including Hindmarsh, Torrens, North Para, Myponga and Light rivers, First and Scott creeks), Murray-Darling NRM region (3 sites including Finniss, Marne and Bremer rivers), Northern and Yorke NRM (2 sites including Hill and Kanyaka rivers) and Kangaroo Island NRM (1 site from Rocky river) (Fig. 1). The macroinvertebrate samples analysed here form part of the Australian Rivers Assessment System (AUSRIVAS) (Davies, 2000) of which South Australia has been part since 1994. The database includes a substantial, standardised

and consistent record on benthic macroinvertebrates and a large number of environmental variables. Annual sampling was conducted in two seasons (autumn and spring), to represent the extremes of variation in physicochemical properties such as temperature, dissolved oxygen and flow, which likely drive differences in biological productivity and biodiversity. We used data collected annually for 13 years from 1994 to 2007 (except 1996 owing to hiatus in national program funding).

Macroinvertebrates were collected using standardised AUSRIVAS protocols which consisted of sampling representative 5 m^2 area of pool habitats within each 100 m study site using a 250 µm mesh square dip net. Sampling involved vigorously kicking the substrate and sweeping the net over a total bank length of 10 m using sequential short sweeping movements at right angles to the bank and, sweeping under overhanging or emergent vegetation (Davies, 2000). Collected macroinvertebrates were preserved in ethanol on site, transported to the laboratory, and subsampled (where 10% of the samples were counted and identified using light and dissecting microscopes), and the residue scanned for rare taxa (Davies, 2000, Simpson and Norris, 2000). This approach ensured observer bias was minimised when counting individuals compared to alternative live-pick approaches included in the AUSRIVAS protocols and it also provided an accurate estimation of the abundance of cryptic taxa. Taxa were identified to the lowest taxonomic level, given available keys, life-history stage and condition. This was most often to genus or species level except for Hydracarina (mites), and some Oligochaeta (worms). Voucher specimens of all taxa were retained as a reference collection at the South Australia Museum and Australian Water Quality Centre (AWQC).

2.3.3 Land use and environmental information

To calculate land-use in the upstream catchment of each site, we used the GIS "Land use South Australia" layer which is based on remote-sensing satellite data [Australian Natural Resources Data Library (ANRDL, 2008)]. Areas of South Australia mostly used for agriculture have been mapped at 1:25 000, whereas the remaining areas have been mapped at 1:100 000. Land-use categories were based on the Australian Land-use and Management (ALUM) classification (based on remote-sensing satellite data compiled in, 2003). Land-use information was derived as percentage (%) of catchment area upstream from each sampling site. We aggregated several land use categories per site (see supporting Table S1 for details). Existing land-use data [(ANRDL, 2008); Fig. 1] showed that conservation areas (formal and

informal reserves) comprised 38% of the land area. Agriculture (mostly cattle and sheep grazing) of varying types and intensity, and to a lesser extent dryland cereal cropping accounted for 53% of land area. The remaining 3% comprised rural residential and urban uses.

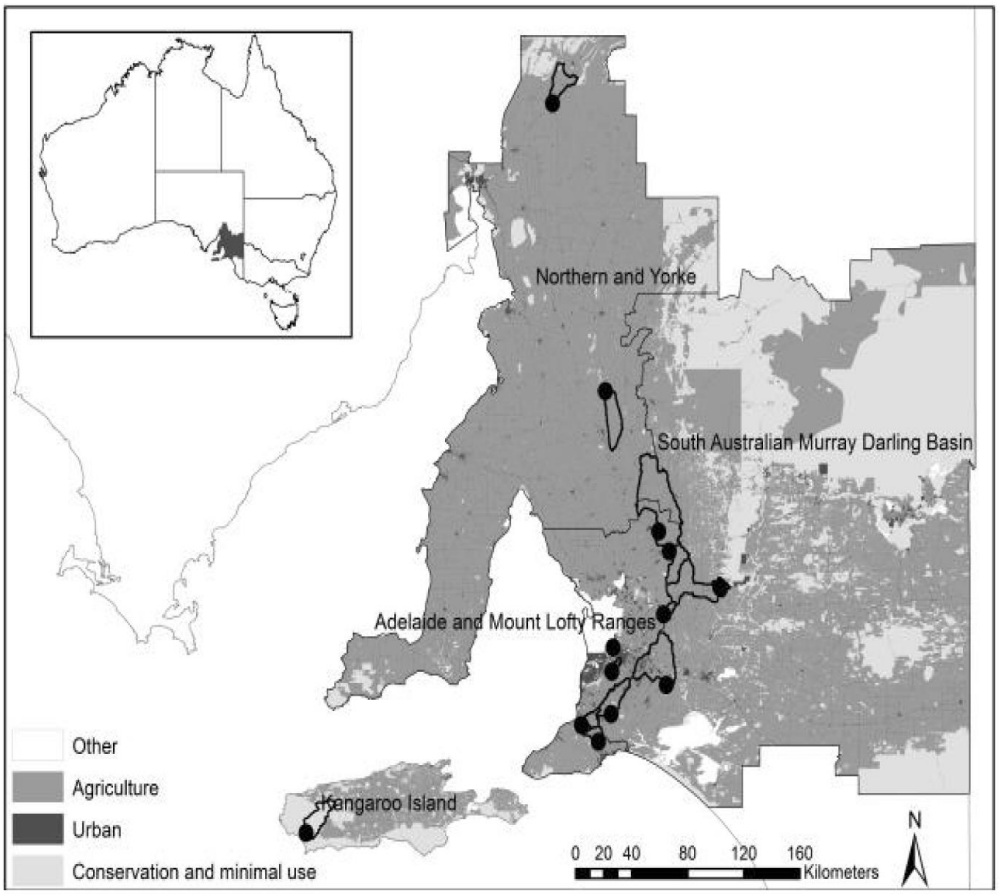

Fig. 1. Map of South Australia, showing the distribution of major land-uses and the sampling sites. Circles represent study sites; heavy black lines represent catchment area upstream of study sites; grey lines represent coastline and state borders; thin black lines represent boundaries to NMR regions. Land-use South Australia layer was sourced from Australian Natural Resources Data Library and their classifications were based on the Australian Land-use and Management (ALUM) classification.

Conductivity, dissolved oxygen and pH were measured *in situ* using calibrated water quality meters (Hach MP-6 portable meter). Work done by Williams (1966 and 1986) in South Australian lakes revealed a very strong relationship ($R^2 = 0.99$) between conductivity and salinity. Additionally, in South Australia, the most prominent influence of agriculture (and water abstraction) is to change stream flow regime, such that greater flow is derived from groundwater which flushes salts (predominantly Na^+ and Cl^- ions) from marine origin into streams (Boulton et al., 2014). Further, there are no secondary sources of salinization such as from mining, coastal inundation, industrial discharge or road de-icing, and electrical

conductivity is dominated by Na+ (median: 72 % of cations) and Cl- (median: 98% of anions), with similar ionic compositions across this region. Therefore conductivity reflects or quantifies salinity alone. We hereby referred to conductivity as salinity. At each site, the physical habitat was characterized as the cover of sand, silt and clay on a scale (0% = no cover, 1-25% = little cover, 26-50% = some cover, 51-75% = moderate cover, 76-100% = extensive cover). Monthly average of estimated local discharge (runoff + drainage) at time of sampling for each stream were sourced from the Australian Water Availability Project (AWAP) (Raupach et al., 2009, Raupach et al., 2012).

2.3.4 Statistical analysis

2.3.4.1 Univariate measures

Analyses were conducted on multivariate (site by taxa abundance matrix) and univariate response variables. Any taxa with fewer than 5 individuals across the 13-year sampling period were considered rare and were excluded from this analysis. The univariate measures were selected based on (Magurran, 2013) and included: (i) Margalef's richness, defined as the total number of different species represented or total number of individuals of all species in the sample. We calculated Margalef's richness using the formula $d = (S\text{-}1) / \log(N)$, where, S is the number of species and N is the abundance or total number of individuals (Margalef, 1958); (ii) Simpson's diversity index, measures the probability that two individuals randomly selected from a sample will belong to the same species. We calculated Simpson's index as $D = 1 - \sum (n_i \times (n_i - 1) / (N \times (N - 1))$, where n_i is the total number of macroinvertebrates of a particular species (the ith taxon) and N is the total number of macroinvertebrates of all species (Simpson, 1949) and; (iii) Pielou's evenness calculated as $J = H / \log(S)$, where, H is Shannon-Weiner diversity and S is the total number of species (Pielou, 1966). All diversity indices were computed using PRIMER-E (v6.1.16) (Clarke and Gorley, 2006), and *R* version 3.2.0 (R Core Team, 2016) with the 'vegan' package (Oksanen et al., 2015).

2.3.4.2 Aim 1: Temporal changes in macroinvertebrate composition

To explore temporal changes in assemblage composition with three factors (site, season and year), permutational multivariate analysis of variance (PERMANOVA) (Clarke and Warwick, 2001) based on Bray-Curtis similarity of fourth-root transformed data (Bray and Curtis, 1957) was used. Site was a nested factor within season and year, and formal testing was undertaken

using the PERMANOVA+ (v1.0.6) software extension to PRIMER (Clarke and Gorley, 2006). Hybrid multidimensional scaling (semi-strong hybrid MDS, threshold = 0.9) (Belbin, 1991) was used to visualize temporal changes in macroinvertebrate assemblages within sites and seasons using the "vegan" package (Oksanen et al., 2015) in R version 3.2.1(R Core Team, 2015). We tested for time (annual) trend of the assemblage trajectories using the seriation test of the RELATE routine in PRIMER-E (Clarke and Gorley, 2006, Somerfield and Gage, 2000). A permutation test (9999 permutations) was used to evaluate significance. The seriation test is based on the assumption that adjacent sample years tend to be closest together in terms of their communities than sample years which are further apart in time (Clarke and Gorley, 2006, Somerfield and Gage, 2000). The test examines the correlations between dissimilarity of communities and time. If $|0.8 \leq \rho \leq 1.0|$ (where, ρ is the Spearman's correlation), then there is a clear trend in the trajectories of the community composition (Clarke and Warwick, 2001), which will be evident by a straight line (a trajectory) in the hybrid MDS plot. Seasonal and annual changes in univariate indices were analysed with generalized linear modelling (GLM) in R.

2.3.4.3 Aim 2: Relationship of macroinvertebrate community composition to environmental, geographic and land use predictors

We used land-use, geographic and environmental variables to examine which variables may be correlated with macroinvertebrate community composition. To explore these relationships, we first examined co-linearity among normalized geographic, environmental and land-use variables using Spearman's correlation coefficient (ρ) and scatter plot matrices to eliminate co-linear variables and reduce redundancy (see supporting Table S2 for details). Variables with the greatest potential ecological importance were used as surrogates for those variables with which they were highly correlated ($|\rho \geq 0.9|$) (Clarke and Warwick, 2001). Distance from source (DFS) and conservation and minimal use (consvMin) (Table S1) were excluded from the analysis because they were highly correlated with catchment area and percentage cover by agriculture respectively. Catchment area or stream size was chosen over DFS because macroinvertebrate species richness has been cited to exhibit strong relationships to catchment area (Heino et al., 2007a, Malmqvist and Maki, 1994). Agriculture was chosen over consvMin because gradients of intensity of agricultural land-use were logically more likely to be associated with changes in assemblage structure from reference/natural condition. Therefore, 12 out of 14 initial candidate variables were used for this analysis. We included

geographic location variables (latitude and longitude) to capture any biogeographic variation in community composition across the large spatial extent of this study.

For the multivariate analysis, a distance-based linear model (DistLM) (Clarke and Gorley, 2006, Warfe et al., 2013) with stepwise regression as selection procedure, using Akaike Information Criterion (AIC) as the selection criterion was used to derive the most parsimonious models predicting macroinvertebrate communities, and for the distance-based redundancy analysis (dbRDA) models. The DistLM enabled us identify predictor variables (on the normalised scale) that contributed significantly to the temporal patterns observed in the assemblage structure as well as determine how much variation was explained by each predictor. The dbRDA plot enabled us visualize the relative contributions of each of the predictor variables on the assemblage structure (Legendre and Anderson, 1999, Magierowski et al., 2012, McArdle and Anderson, 2001). For the univariate analysis, a stepwise regression methods using AIC as the selection criterion was again used to derive the most parsimonious models for each univariate measure. Diagnostic analysis using Variance inflation factors (VIF) were employed to examine how much multicollinearity (correlation between predictors) exists in the multiple regression analysis. None of the VIF inspected exceeded 2.5, so the partial regression coefficients likely provided reliable estimates of effects of each predictor variable while holding the effects of all other variables constant (Berk, 2003).

2.3.4.4 Candidate taxa that correlate with gradients of land-use, environmental or geographic variables.

To examine which taxa were correlated to gradients of the significant predictors identified by DistLM in the Aim 2, we used the BVSTEP (Best Subset of Environmental Variables with maximum Correlation with Community Dissimilarities) procedure in PRIMER (Clarke and Gorley, 2006, Clarke and Warwick, 2001) . This procedure finds subsets of taxa (vulnerable and opportunistic taxa) which are best correlated with the patterns in the predictor variables (on a distance matrix between predictors) (Clarke and Ainsworth, 1993). A permutation test (9999 permutations) was used to evaluate the significance of the results. Individual Spearman's rank correlations (ρ) were then used to evaluate the direction and strength of the relationships between each taxon and each predictor variable. We define indicator taxa as those which were significantly ($P \leq 0.05$) correlated with the predictor variables.

2.4 Results

We collected 338 samples which comprised 173,149 individuals from 840 taxa. Autumn samples comprised 66,503 individuals while 106,646 individuals were recorded in spring.

2.4.1. Aim 1: Temporal change in assemblage composition

Multivariate analysis showed that macroinvertebrate community composition differed significantly among sites (Table 1), as did the shape of their trajectories (Table 2, Fig. 2). Within sites, macroinvertebrate communities varied between autumn and spring and differed among the years (Table 1), but there was no clear trend in their trajectories within sites across years (as indicated by $\rho < 0.8$ from the RELATE procedure) (Table 2, Fig. 2). There was no indication that sites changed in any consistent way among years (Fig. 2).

Univariate analysis based on species richness, evenness and diversity indicated significant differences among sites (Table 3). Richness did not differ across seasons but varied significantly across the years. Within sites, Evenness was higher in autumn (0.63 ± 0.01) than in spring (0.58 ± 0.01) but did not differ among years. Diversity was also higher in autumn (0.76 ± 0.01) than in spring (0.72 ± 0.02), but also did not differ among years. The GLMs explained more of the variation in richness than evenness or diversity (Table 3).

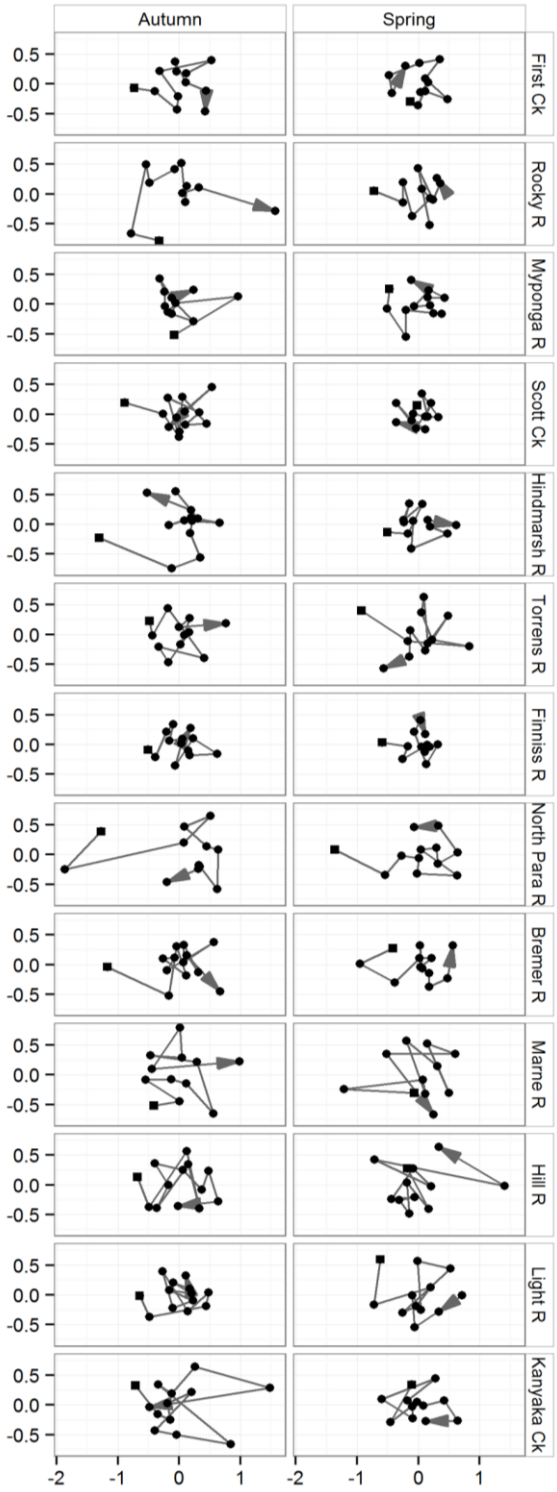

Fig. 2. **Unconstrained ordination plots of macroinvertebrates in autumn and spring**. Unconstrained (semi-strong hybrid MDS) ordination plots of macroinvertebrates (individual sites across years) based on Bray-Curtis similarity of 4th root abundance data in autumn and spring. Sites names with "Ck" and "R" represent creeks and rivers respectively. The lines connecting the dots represent trajectories of assemblage structure across the years. Square symbols indicate the start of the trajectory and the arrow head indicates the end of the trajectory. The scale represents dissimilarity of sites.

Table 1: Results of permutational multivariate analysis of variance (PERMANOVA). *df* represents degrees of freedom. Bold numbers indicate significant *P*-values

	df	***F***	***P***
Site	12	4.02	< **0.001**
Season(Site)	13	2.48	< **0.001**
Year(Site)	155	1.40	< **0.001**
Residual	136		

Table 2: RELATE results (ρ and *P*-value) reported for seriation of macroinvertebrate composition at each site for each season. Sites names with "Ck" and "R" represent creeks and rivers respectively. ρ signifies Spearman's correlations in the seriation test; if $|0.8 \leq \rho \leq 1.0|$, then there is a clear trend in the trajectories of the community composition (Clarke and Warwick, 2001).

Site	Autumn ρ	Spring ρ
First Ck	0.41	0.30
Rocky R	0.55	0.38
Myponga R	0.34	0.48
Scott Ck	0.16	0.22
Hindmarsh R	0.45	0.41
Torrens R	0.37	0.32
Finniss R	0.37	0.35
North Para R	0.47	0.49
Bremer R	0.34	0.56
Marne R	0.29	0.12
Hill R	0.17	0.27
Light R	0.32	0.21
Kanyaka Ck	0.12	0.20

Table 3: Results of general linear models for the relationships of the biodiversity indices to site, season (site nested within season) and year (site nested within year).

	df	MS	*F*	*P*
Richness (R^2 = 0.528)				
Site	12	37.74	19.69	< **0.001**
Season(Site)	13	2.04	1.06	0.392
Year(Site)	13	8.94	4.67	< **0.001**
Residuals	278	1.92		
Evenness (R^2 = 0.355)				
Site	12	0.16	9.06	**0.001**
Season(Site)	13	0.05	2.93	< **0.001**
Year(Site)	13	0.01	0.46	0.947
Residuals	278	0.02		
Diversity (R^2 = 0.310)				
Site	12	0.16	6.83	< **0.001**
Season(Site)	13	0.06	2.84	**0.001**
Year(Site)	13	0.01	0.39	0.972
Residuals	278	0.02		

df represents the degrees of freedom for the sources of variation. Bold numbers indicate significant *P*-values.

2.4.2 Aim 2: Relationships of macroinvertebrate community composition to environmental, geographic and land-use predictor variables

Since multivariate analysis demonstrated that community structure differed between seasons (Table 1), we evaluated them separately for the remaining analyses. In autumn, 8 out of the 12 predictor variables explained significant amounts (total of 23.7%) of the variability in community composition (Table 4, Fig. 3). Conductivity was most strongly related (explaining 6.8% of the total variation in the assemblage structure) to the community structure, followed by latitude (3.4%) and agriculture (3.1%). Macroinvertebrate communities among sites in autumn were more closely clustered together (Fig. 3) relative to spring (Fig. 4).

Table 4: Results from a distance-based linear model (DistLM) for the 13 sites in autumn and spring**.** Variables are listed in order of contribution to explaining variation in the community composition. % variation represents explained variation attributable to each variable added to the model. Abbreviations for predictor variables are listed in Table S1.

Variable	*F*	*P*-value	% variation
Autumn (R^2 = 23.7; AIC = 1275.9)			
Cond	11.71	< **0.001**	6.82
Latitude	5.93	< **0.001**	3.35
Agric	5.55	< **0.001**	3.05
Urban	5.03	< **0.001**	2.69
CatchArea	4.97	< **0.001**	2.60
Longitude	4.66	< **0.001**	2.38
Detc	3.36	< **0.001**	1.69
FineSed	2.33	< **0.001**	1.16
Spring (R^2 = 27.3; AIC = 1208.1)			
Cond	14.66	< **0.001**	8.74
Latitude	7.97	< **0.001**	4.54
Longitude	5.94	< **0.001**	3.28
Agric	5.01	< **0.001**	2.70
CatchArea	4.99	< **0.001**	2.61
Urban	3.83	< **0.001**	1.97
Runoff	3.04	< **0.001**	1.54
FineSed	2.83	< **0.001**	1.41
Detc	1.82	< **0.001**	1.41

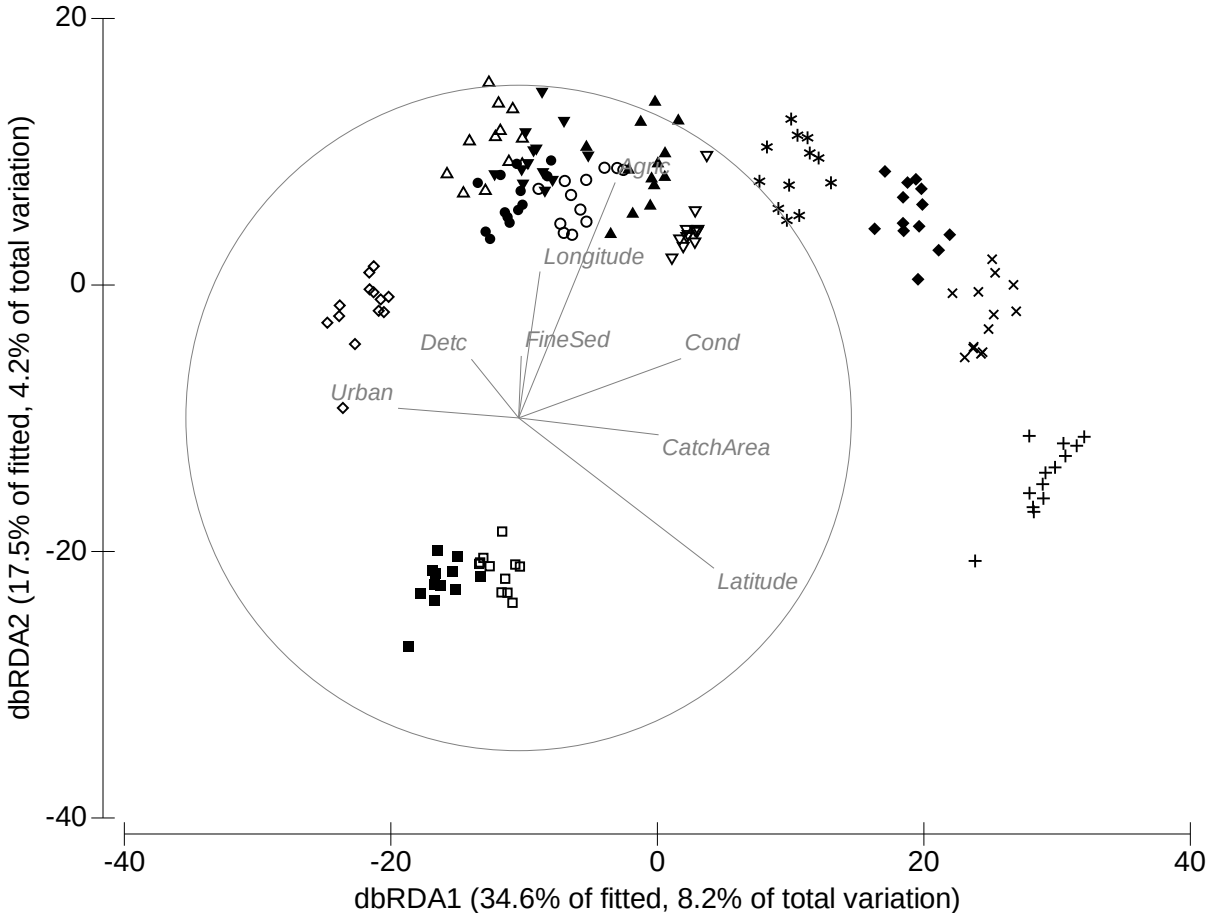

Fig. 3. Distance-based redundancy analysis (dbRDA) of macroinvertebrate samples in autumn. Distance-based redundancy analysis (dbRDA) of macroinvertebrate samples in autumn, overlaid with normalised predictor variables (based on distLM analysis in Table 4). Abbreviations for predictor variables are listed in Table S1.

In spring, 9 out of the 12 predictor variables explained significant amounts (total of 27.3%) of the variability in community composition (Table 4, Fig. 4). Again, conductivity was most strongly related (8.7% of the total variation in the assemblage structure) to community structure, followed by latitude (4.5%) and longitude (3.3%). Sites were distinct in their community composition in spring (Fig. 4) relative to autumn (Fig. 3).

A number of variables (conductivity, latitude, agriculture and urban land-uses, catchment area, longitude, detritus cover and fine sediments) were consistently predictive of macroinvertebrate community structure in both seasons. Predictor variables explained more of the variation in community structure in spring than in autumn (Table 4).

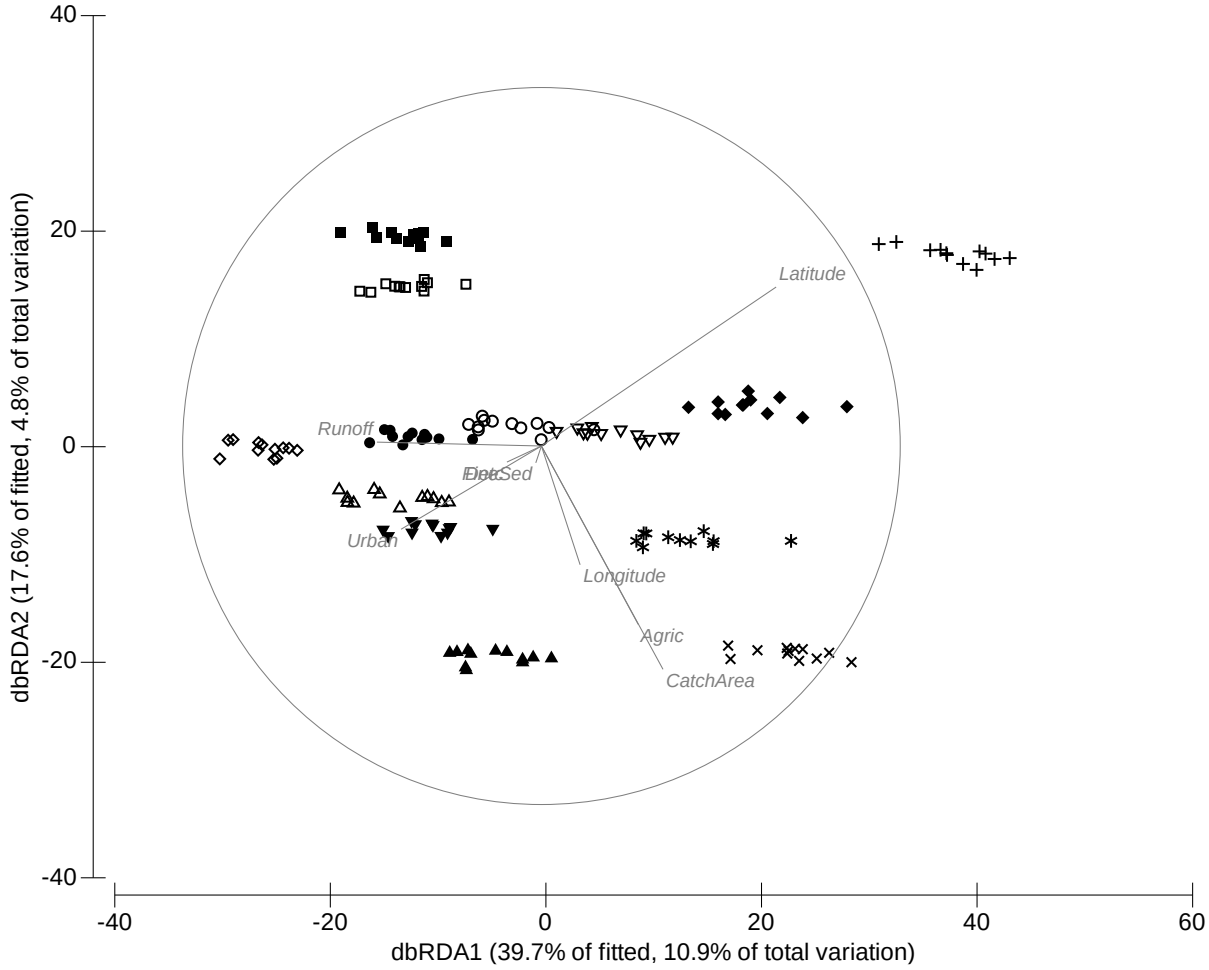

Fig. 4. Distance-based redundancy analysis (dbRDA) of macroinvertebrate samples in spring. Distance-based redundancy analysis (dbRDA) of macroinvertebrate samples in spring, overlaid with normalised predictor variables (based on distLM analysis in Table 4). Abbreviations for predictor variables are listed in Table S1.

Results from the univariate analyses showed that during autumn, richness was positively related with increased cover by agriculture and urban land-uses as well as detritus cover, but reduced with conductivity, longitude, and fine sediments (Table 5). Evenness was positively related with increased cover by only urban land-use (Table 5). Diversity was also positively related with increased cover by urban land-use and declined with conductivity. Urban land-use was a predictor across all 3 univariate measures used in the analysis. The stepwise regression models explained more of the variation in richness than evenness or diversity.

Table 5: Results of general linear models for the relationships of the biodiversity indices to geographic, environmental and land-use predictor variables in autumn and spring. S.E. represents the standard error of the coefficients. Bold numbers indicate significant P-values. *indicates trending *P*-values. Abbreviations for predictor variables are listed in Table S1.

Autumn				**Spring**			
Variables	Coefficient ± S.E.	*t*- value	*P*-values	Variables	Coefficient ± S.E.	*t*- value	*P*-values
	Richness (R^2 = 0.46)				**Richness (R^2 = 0.36)**		
Intercept	7.36 ± 1.72	4.29	< **0.001**	Intercept	9.42 ± 1.16	8.13	< **0.001**
Long	-0.68 ± 0.36	-1.92	*0.057	Long	-0.87 ± 0.38	-2.30	**0.023**
Agric	1.59 ± 0.56	2.84	**0.005**	Agric	1.44 ± 0.67	2.14	**0.034**
Urban	5.50 ± 1.35	4.09	< **0.001**	Urban	6.29 ± 1.45	4.34	< **0.001**
Cond	-1.02 ± 0.15	-6.78	< **0.001**	Cond	-0.85 ± 0.16	-5.24	< **0.001**
PH	0.38 ± 0.23	1.65	0.100				
FineSed	-0.63 ± 0.26	-2.42	**0.017**		**Evenness (R^2 = 0.17)**		
Detc	1.40 ± 0.43	3.27	**0.001**	Intercept	1.08 ± 0.16	6.68	< **0.001**
				Lat	0.04 ± 0.02	1.84	*0.067
	Evenness (R^2 = 0.08)			Runoff	-59.76 ± 35.38	-1.69	*0.093
Intercept	0.57 ± 0.02	29.55	< **0.001**	Urban	0.26 ± 0.10	2.50	**0.014**
Urban	0.27 ± 0.07	3.80	< **0.001**	Cond	-0.07 ± 0.02	-3.85	< **0.001**
				FineSed	0.04 ± 0.03	1.70	*0.091
	Diversity (R^2 = 0.21)						
Intercept	2.75 ± 0.31	8.80	< **0.001**		**Diversity (R^2 = 0.26)**		
Urban	1.28 ± 0.29	4.35	< **0.001**	Intercept	3.86 ± 0.51	7.56	< **0.001**
Cond	-0.11 ± 0.04	-3.10	**0.002**	Lat	0.15 ± 0.08	2.06	**0.041**
				Urban	1.13 ± 0.36	3.14	**0.002**
				Cond	-0.29 ± 0.06	-4.59	< **0.001**
				FineSed	0.14 ± 0.09	1.52	0.132

During spring, richness was positively related with increased cover by agriculture and urban land-uses but declined with longitude and conductivity. Evenness was positively related with increased urban land-use but negatively related with conductivity. Evenness exhibited trends of decline with runoff but exhibited increasing trends with fine sediment and latitude (Table 5). Diversity was positively related with increased cover by urban land-use and latitude but declined with conductivity. Urban land-use and conductivity were predictors across all three univariate biodiversity indices used in the analysis. The stepwise regression models explained more of the variation in richness than evenness or diversity (Table 5).

A small number of variables were consistently predictive of univariate indices in both seasons. Conductivity, agriculture and urban land-uses were significantly associated with richness in autumn and spring, but only urban land-use was predictive of evenness in both seasons. Conductivity and urban land-use were predictive of diversity in autumn and spring.

2.4.3 Aim 3: Candidate taxa that correlate with gradients of particular land-use, environmental and geographic determinants.

Out of a total of 840 macroinvertebrates taxa used in the analysis, 14 taxa were significantly correlated with the strongest predictors of community composition in autumn (Mantel's test: $P < 0.001$, $\rho = 0.43$) (Table 6). Out of these 14 taxa, 13 were all negatively correlated with conductivity and 11 taxa were all negatively correlated with latitude. The abundance of Dixidae and *Physa acuta* was negatively correlated with catchment area (stream size) and latitude respectively. Only one taxon (*Aphroteniella* sp.) was correlated with gradient of conductivity, cover by agriculture and urban land-uses, catchment area, longitude and detritus cover. The abundance of *Aphroteniella* sp. was negatively correlated with agriculture and stream size but positively related to cover by urban land-use (Table 6).

A number of taxa were also weakly ($|-0.4 \leq \rho \leq -0.1|$; $|0.1 \leq \rho \leq 0.4|$) and moderately ($\rho = 0.5$, $\rho = -0.5$) correlated with the predictor variables (Table 6). Taxa that correlated with more than one predictor variables were common.

Table 6: Macroinvertebrates indicated by BVSTEP as associated with gradients of specific predictor variables in autumn and spring. Numbers written in the cells are Spearman's correlation values between the taxon and gradient of that predictor variable. Predictor variables for both seasons are arranged in the order in which the most influential variables in each season appear as indicated by DistLM (Table 4) appear. Abbreviations for predictor variables are listed in Table S1.

Autumn

Taxa	Family	Class/ Order	Cond	Lat	Agric	Urban	CatchArea	Long	Detc	FineSed
ρ			0.50	0.47	0.35	0.30	0.35	0.35	0.10	0.14
***P*-value**			**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.151	**0.018**
Physa acuta	Physidae	Gastropoda	-0.3	-0.5						
Nais sp.	Naididae	Oligochaeta	-0.4	-0.3						
Chaetogaster	Naididae	Oligochaeta	-0.2	-0.3						
Tipulidae	Tipulidae	Diptera	-0.2	-0.3						0.2
Dixidae	Dixidae	Diptera	-0.3	-0.1	-0.4		-0.5	$1x10^{-9}$		
Aphroteniella sp.	Aphroteniinae	Diptera	-0.4	$1x10^{-12}$	-0.4	0.4	-0.4	0.1	-0.1	
Riethia sp.	Pseudochironomini	Oligochaeta	-0.3	-0.2						
Tasmanocoenis tillyardi	Caenidae	Ephemeroptera	-0.4	-0.2						
Sigara sp.	Corixidae	Hemiptera	-0.2	-0.3						
Newmanoperla thoreyi	Gripopterygidae	Plecoptera	-0.2							
Hellyethira simplex	Hydroptilidae	Trichoptera	-0.3	-0.3						
Oxyethira columba	Hydroptilidae	Trichoptera	-0.2	-0.2						
Lingora aurata	Conoesucidae	Trichoptera								
Leptorussa sp.	Leptoceridae	Trichoptera	-0.2	-0.3						0.2

Spring

Taxa	Family	Order	Cond	Lat	Long	Agric	CatchArea	Urban	Runoff	FineSed	Detc
ρ			0.44	0.51	0.36	0.31	0.27	0.35	0.36	0.19	0.16
***P*-value**			**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.004**	**0.009**
Ferrissia petterdi	Ancylidae	Gastropoda		-0.3					0.2		
Physa acuta	Physidae	Gastropoda	-0.2	-0.3					0.3		
Nais sp.	Naididae	Oligochaeta		-0.1					0.4		
Paranais litoralis	Naididae	Oligochaeta									
Gammarus sp.	Eusiridae	Amphipoda									
Eusiridae	Eusiridae	Amphipoda		0.3							
Perthiidae	Perthiidae	Amphipoda						0.3			
Necterosoma penicillatus	Dytiscidae	Coleoptera		0.4				-0.3			
Dixidae	Dixidae	Diptera			$1x10^{-16}$						
Empididae	Empididae	Diptera	-0.4								

Larsia sp.	Chironomidae	Diptera						-0.3			
Corynoneura sp.	Orthocladiinae	Diptera							0.4		
Riethia sp.	Pseudochironomini	Oligochaeta	-0.4	-0.3							
Stempellina sp.	Chironominae	Diptera		-0.3							
Leptophlebiidae	Leptophlebiidae	Ephemeroptera	-0.4	-0.3					0.5		
Tasmanocoenis tillyardi	Caenidae	Ephemeroptera	-0.4	-0.2					0.4		
Micronecta sp.	Corixidae	Hemiptera									-0.3
Anisops sp.	Notonectidae	Hemiptera						-0.3			
Austrolestes annulosus	Lestidae	Odonata									0.2
Diplacodes haematodes	Libellulidae	Odonata		0.3							
Orthetrum caledonicum	Libellulidae	Odonata									
Libellulidae	Libellulidae	Odonata		0.3							
Newmanoperla thoreyi	Gripopterygidae	Plecoptera	-0.4			-0.5			0.3		
Gripopterygidae	Gripopterygidae	Plecoptera	-0.3								0.1
Austrocerca tasmanica	Notonemouridae	Plecoptera	-0.4	-0.2	-0.1				0.5		
Hydrobiosidae	Hydrobiosidae	Trichoptera									
Oxyethira columba	Hydroptilidae	Trichoptera	-0.3	-0.4	-0.4	-0.4	0.1	-0.4		0.3	
Lingora aurata	Conoesucidae	Trichoptera									
Atriplectides dubius	Atriplectidae	Trichoptera									0.1
Lectrides varians	Leptoceridae	Trichoptera	-0.5						0.4		
Leptorussa sp.	Leptoceridae	Trichoptera						-0.2			
Notalina bifaria	Leptoceridae	Trichoptera									$1x10^{-11}$

ρ signifies Spearman's correlations and *P*-value shows the significance of the relationship between the macroinvertebrates and the predictor variables. Blank cells between taxon and predictor variable indicate that taxon was not correlated with that predictor variable.

In spring, 32 taxa were correlated (Mantel's test: $P < 0.001$, $\rho = 0.59$) with the most related predictors of macroinvertebrate community composition (Table 6). Ten of these taxa were significantly correlated with conductivity. The abundance of all these taxa declined with increasing conductivity. A total of 9 taxa were correlated with runoff. The abundance of all these taxa increased with increasing runoff. Two and 6 taxa were significantly correlated with agriculture and urban land-uses respectively. The abundance of *Newmanoperla thoreyi* was negatively correlated with cover by agriculture land-use (Table 6). A number of taxa were weakly ($|-0.4 \leq \rho \leq -0.1|$; $|0.1 \leq \rho \leq 0.4|$) and moderately ($\rho = 0.5$, $\rho = -0.5$) correlated with the predictor variables. Taxa correlated with greater than one predictor variables were common. Nine taxa were common to both seasons.

2.5 Discussion

Our study sought to determine the most influential environmental variable driving the taxonomic community composition, in addition to known importance of flow. Using a 13 year dataset, we found the following: (1) temporal trajectories of macroinvertebrate communities in temporary streams varied within sites in both seasons and across the years. Temporal trajectories of macroinvertebrate communities differed between sites but there was no consistent trend in the trajectories within sites across years; (2) a combination of land-use, geographic and environmental variables accounted for 24% of the variation in the community structure in autumn and 27% in spring; (3) in autumn 14 taxa were significantly related to the most related predictors of community structure across sites. In contrast, during spring, 32 taxa were significantly related to the most related predictors of community structure. Our results indicate that temporal variability of macroinvertebrates in these temporary streams is predicted significantly (but modestly) by a combination of factors but most strongly and consistently related to conductivity, longitude, latitude and the proportion of catchment under agricultural and urban land-uses.

2.5.1 Temporal changes in macroinvertebrates composition

Macroinvertebrate community composition varied among individual sites across the years. Given the variability in the biophysical variables among years, this result was unsurprising. These differences may also have been observed because of differences in the ability for macroinvertebrates to have survived at each site (Lake et al., 2007). Similarly, a study by Leigh and Sheldon (2009) also found high temporal variability of macroinvertebrates in

Australian dryland rivers. Our results may indicate that stochastic processes such as climate variability or differential dispersal abilities of macroinvertebrates may be important (Blanchette and Pearson, 2013). Community composition also varied seasonally within sites. Sites in autumn were more similar in their community composition than sites in spring. The strongest correlates of community structure in autumn included all the predictor variables used in the analysis except runoff, fine sediments, algal cover, dissolved oxygen and pH, whereas in spring fine sediment and runoff were also related to community structure. The differences in community composition between seasons may be due to the fact that different taxa show differential success between seasons according to their particular resilience or resistance traits (Blanchette and Pearson, 2013).

We predicted that during summer (characterized by high temperature, little or no flows), macroinvertebrates diversity would decline relative to wetter, cooler spring conditions. However, we found that within the same site, diversity and evenness were significantly higher in autumn than in spring whereas richness did not vary between the two seasons. This distinction in diversity and richness among the two seasons could also be due to the combination of the multiple physical factors and the inherent flow variability that characterize temporary streams.

2.5.2 Relationship of macroinvertebrate community composition, environmental, geographic and land-use predictor variables

Our study has shown that conductivity was the most consistent predictor of assemblage composition in both autumn and spring. Conductivity alone was associated with more variation in macroinvertebrate assemblage structure than any other land-use, geographic or environmental variable. These responses are reflected in the declines in richness, evenness and diversity with conductivity in both seasons. The relationship is broadly consistent with earlier studies describing salinity as a major driver of community composition (Carver et al., 2009, Kefford et al., 2011, Pinder et al., 2005). We therefore propose that salinity exerts a strong direct pressure on macroinvertebrate assemblages in temporary streams by selecting for saline-tolerant taxa, while selecting against the more halo-sensitive taxa, thus leading to general declines in richness, evenness and diversity.

Our results showed that, in autumn, macroinvertebrate communities among sites in these temporary streams were more closely clustered together relative to spring, suggesting

that community composition was more similar in autumn than in spring. This difference in assemblage composition between the two seasons may be likely due to differences in flow variability that characterize these temporary streams (Sheldon and Thoms, 2006). Our study showed that agriculture and urban land-uses were also significant predictors of community structure in autumn and spring, with agriculture being the most related land-use predictor. Richness, evenness and diversity were strongly correlated with the land-use variables in both seasons. These responses were reflected in the declines in richness, evenness and diversity with agricultural land-use. These relationships were broadly consistent with earlier studies describing changes in macroinvertebrate communities in agricultural catchments (Collier, 2008, Magierowski et al., 2012). These patterns may be driven by multiple mechanisms common to all agricultural land-use (Steel et al., 2010), such as changes in water quality (including enrichment of nutrients and increases in salinity and temperature), lack of riparian zones and dominance of fine sediments (Collier, 2008, Magierowski et al., 2012, Reid et al., 2013).

Our results showed that geographic location variables (latitude and longitude) were predictive of community structure in both autumn and spring. To some extent, decreasing latitude in this region is correlated with increasing dryness and increasing salinity (see Table S2), while Longitude may reflect the rain-shadow effect of the Mount Lofty Ranges in part of the study region. Richness, evenness and diversity were significantly correlated with the location variables in both seasons. These relationships were broadly consistent with earlier studies describing spatial variability of macroinvertebrate communities among different sites (locations) (Marshall et al., 2006, McRae et al., 2004). Our results indicate that understanding biogeography of community structure is important for conservation because different sites at large spatial scales harbor different components of the regional assemblage and this variation is not captured by considering conductivity alone.

2.5.3 Candidate taxa that correlated with gradients of particular land-uses, environmental and geographic variables.

When streams are disturbed, taxa that are sensitive to those stressors will be eliminated, leaving communities to be dominated by only taxa that are resistant (able to survive the impacts) or resilient (have efficient recovery mechanisms). Our results showed that in both seasons, a number of taxa were weakly to moderately correlated with the strongest correlates of community structure. The reason for these weak to moderate correlations might be due to

the sparseness of most taxa recorded and the finer taxonomic resolution (genus and species) we used in our analysis. Furthermore, it was common that a taxon, which was correlated with a single predictor variable, also responded significantly to other predictor variables. This may be due to the autocorrelations that existed among the predictor variables used in our analysis. Underwood and Peterson (1988) described indicator taxa as those taxa that are highly correlated with a predictor variable of interest and not correlated with any other predictor variable. Under this definition, no indicator taxa were evident in autumn and 10 indicator taxa (Perthiidae, Empididae, *Corynoneura sp*., *Micronecta sp*., *Anisops sp*., *Austrolestes annulosus*, *Diplacodes haematodes*, Libellulidae, *Atriplectides dubius*, *Leptorussa sp*.) were recorded in spring. Since these "indicator taxa" provided a poor representation of the overall variability (and were also weakly correlated with the predictor variables of interest), alternative approaches to identifying indicators (e.g. trait-based approaches) (Mason and de Bello, 2013) may provide additional information useful for condition assessment in temporary streams.

2.6 Conclusions

When temporary streams become impacted with harsh natural or anthropogenic conditions, macroinvertebrate communities tend to become more similar because the tolerant macroinvertebrate generalists dominate (Sheldon, 2005). The differential colonization and survival of macroinvertebrates in this study highlights the importance of local factors in structuring macroinvertebrate communities, particularly conductivity, location, and the extent of agricultural and urban land-uses. The highly variable nature of temporary streams, coupled with the site-specific changes in macroinvertebrate assemblages, pose a challenge when developing monitoring programmes and managing such waters (Sheldon, 2005). The effects of anthropogenic degradation may mimic natural declines in species abundance and diversity, which are related to seasonal recession of temporary streams, creating difficulties in separating changes due to human impact from those due to natural processes. Although we provide an improved understanding of the temporal variability in assemblage composition of intermittent streams, the extreme variability we found using taxonomically-based metrics presents an even more challenging scenario for monitoring. Alternative approaches to biomonitoring using traits may provide additional information useful for measures of the conditions in temporary streams (Chessman et al., 2010, Chessman and Royal, 2004).

2.7 Acknowledgements

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2.8. Author contributions

Conceived and designed the experiments: PKB SC LAB RM. Performed the experiments: PG PM. Analyzed the data: PKB RM LAB SC. Wrote the paper: PKB. Commented on previous versions of the manuscript: SC RM LAB PG PM.

2.9 Supporting information

Table S1 List of environmental, geographic and land use predictor variables**.**

Table S2 Spearman's correlations coefficients (ρ) between environmental, geographic and land-use predictor variables for the 13 sites. Abbreviations for predictor variables are listed in Table S1. Bold numbers indicate $\rho > 0.90$ between variables for which reason one variable for chosen as a surrogate for the other variable.

Table S3 List of 13 sites surveyed in this study.

Chapter 3

Response of macroinvertebrate traits to flow and salinity in stream ecosystems: a synthesis

Mayfly (Photo by Mick Hall)

This chapter is in review in *Hydrobiologia*:

Botwe, P. K., Barmuta, L. A., Magierowski, R., McEvoy, P., Goonan, P. & Carver, S. Response of macroinvertebrate traits to flow and salinity in stream ecosystems: a synthesis.

3.1 Abstract

Freshwater ecosystems are commonly affected by flow and salinity, and both these stressors are known to affect macroinvertebrate traits individually, but how consistent study findings are has not yet been synthesized. Here, we fill this gap by providing a synthesis of trait responses to effects of flow and salinity, to examine if generalisations can be drawn about these effects. We examined the spatio-temporal extents of research into effects of flow and salinity, methodological approaches used, and which traits responded consistently to flow and salinity and which do not. We found that, the number of publications on macroinvertebrate trait responses to flow and salinity are accumulating at a slow, but steady rate, with majority of studies being conducted in Europe, Oceania and North America. Further, our results showed heterogeneity in responses of traits to effects of flow and salinity, which may be partly due to differences in methodological approaches among studies. To improve the diagnostic ability of bioassessment based on species traits, there is the need for consolidated and comprehensive trait databases resolved at genus or species levels, standardized in terms of trait classifications and nomenclature, because contrasting results among studies may reflect not only ecological but methodological differences as well.

3.2 Introduction

Flow and elevated salinity are major drivers of macroinvertebrate communities in dry environments globally (Williams, 2002). The effects of these drivers have traditionally been assessed separately using taxonomic methods (macroinvertebrates identities, abundance and distribution) (Booker et al., 2015, Canedo-Arguelles et al., 2012). While valuable, limitations of this literature is the consideration of the interactions between flow and salinity (Brock et al., 2005). Further, these taxonomic studies are restricted in its extendibility across studies owing to biogeographic variation in species identities across different regions (Townsend et al., 1997). Therefore, assessments using invertebrate traits have been proposed to help overcome taxonomic limitations, improve our understanding of the structure and dynamics of ecological communities, and potentially predict their response to natural or anthropogenic disturbances (McGill et al., 2006). However, there is no consensus regarding the effects of flow and salinity on macroinvertebrate trait composition.

Here, we systematically review the literature on effects of flow and salinity on macroinvertebrate traits, and examine whether generalisations can be drawn about these

effects. While it is recognized that salinity (Szöcs et al., 2014) and flow (Walters, 2011) can have significant effects on macroinvertebrate traits, results among studies are not always consistent. For instance, Szöcs et al. (2014) found that increasing salinity was positively correlated with the proportion of shredders, while Kefford et al. (2012) reported a decrease in the number of shredders in higher salinities. Walters (2011) reported that during low flows, the proportion of taxa with small body size decreased, but Bêche and Resh (2007) reported the opposite response. Indeed these inconsistencies raise questions as to whether existing information in the literature can be used to make broad generalisations about macroinvertebrate traits response to effects of flow and salinity.

To address these issues, we reviewed the trait-based literature to answer the following: (1) what have been the temporal and geographic extents of research into effects of flow and salinity on macroinvertebrate traits? (2) What have been the methodological approaches used? 3) Which traits respond consistently to flow and salinity, and which do not?
This review presents an important step by incorporating an expansive view of past research efforts. By quantifying past spatial and temporal extent of research, as well as methodological approaches, we highlight research gaps and suggest directions for future studies. Most importantly, this review is the first to comprehensively and critically integrate research on traits response to effects of flow and salinity.

3.3 Methods

3.3.1 Literature search and publication screening

A systematic literature search was conducted in May 2016 using Web of Science™ Core Collection. Keywords were chosen to target studies examining macroinvertebrate traits response to effects of salinity and flow in stream ecosystems (Appendix 1). The search was restricted to peer-reviewed journal articles relevant to aquatic macroinvertebrates. Resulting publications were exported into Endnote vX7.7 and duplicates removed. The remaining studies were screened for inclusion based on the following criteria: 1) they were published in English, 2) their full text was available (e.g. conference abstracts were excluded), 3) they were original empirical research (e.g. review papers were excluded) and 4) they reported the effects of salinity or flow on macroinvertebrate biological traits in streams. We did not conduct a meta-analysis because the studies were too divergent in their methodological and

analytical approaches, to allow for valid mathematical combinations (or quantitative analysis) of results.

3.3.2 Data collection

For the selected publications, data were extracted for: year of publication, authors, title of article, name of journal, country and continent where research was conducted, duration of study, spatial scale of study (longitudinal, catchment, or multiple catchments), methods of statistical analysis (how traits were linked to environmental conditions), and the macroinvertebrate traits which responded to flow and salinity as well as the direction of response (increase or decrease).

We categorized high flow events into two: high flows as a result of floods, and high flows resulting from flow intermittency. A flood high flow here refers to mean daily flow exceeding 220 m^3/S, while a high flow refers to discharge > 75^{th} percentile mean daily flow (Caruso et al., 2013). Intermittent high flow and normal flow in non-intermittent stream were categorised together. Similarly, low flow event was categorised into low flows resulting from drought, and low flows resulting from flow intermittency. We also categorized salinity-trait studies into high salinity (4,000 µS/cm ≤ salinity ≤ 130,084 µS/cm) and low salinity (0µS/cm < salinity < 4,000 µS/cm). While there are some salinity classifications developed for lakes (Millan et al., 2011), there are no such conventional classifications yet developed for rivers and streams (i.e. flowing water), unlike metrics developed for flow. Therefore, we categorised salinity based own how the various studies used in our systematic review have described the range of the salinity values. Generally, studies described salinity with range of values 0µS/cm < salinity < 4,000 µS/cm as low, and 4,000 µS/cm ≤ salinity ≤ 130,084 µS/cm as high salinity, and this was consistent across the studies used in our systematic review.

3.4 Results

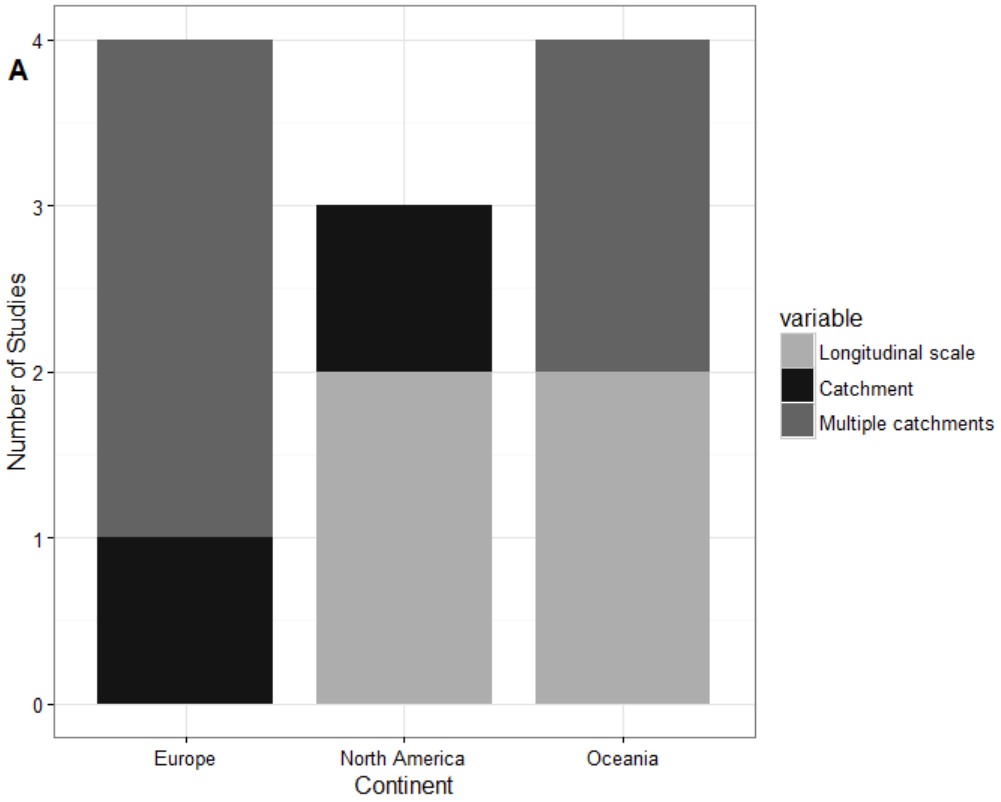
A total of 36 studies (after excluding duplicates) were initially generated from the database search spanning the years 1945 to 2016. However, when we applied the inclusion criteria, 11 papers were flow related and seven were related to salinity, giving a total of 18 papers [spanning 2006 to 2016 (Table 1, Appendix 2)]. Only one non-English study was found and was excluded from the 36 studies.

3.4.1 Temporal and geographic scope

Studies of flow-trait relationships have varied from zero to two papers published per year, while there have been fewer studies on salinity (maximum of 1 per year: Table 1). Flow studies were geographically dispersed with four studies conducted in Europe, four in Oceania and three in North America (Fig. 1A, Appendix 2). One of the four European studies was conducted at catchment scale while the remaining three were across multiple catchments. In North America, two studies were conducted at spatial longitudinal scales (i.e. multiple sites were sampled across a stretch of river) and one at catchment scale. In Oceania, two studies were conducted at spatial longitudinal scales and two across multiple catchments. Studies of salinity were more geographically clustered with five studies in Europe and two in Oceania (Fig. 1B, Appendix 2). Three of the European studies were conducted at spatial longitudinal scales and the remaining two at catchment scales. Both Oceania studies were conducted across multiple catchments.

Table 1. Number of studies published regarding flow-trait and salinity-trait relationships. "N" represents the total number of studies

Year	Number of studies	
	Flow	Salinity
2006	1	1
2007	2	0
2008	1	1
2009	0	1
2010	1	0
2011	2	1
2012	0	1
2013	1	1
2014	0	1
2015	1	0
2016	2	0
	N = 11	N = 7



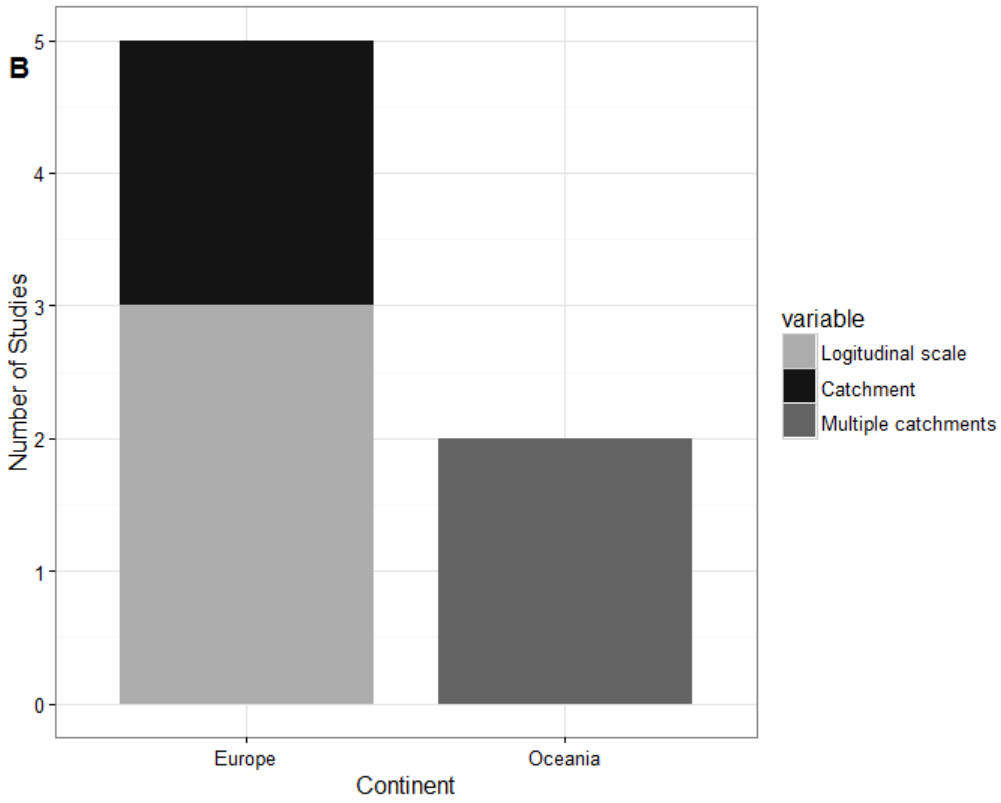


Fig 1. Geographic scope of study regarding macroinvertebrate traits response to effects of A) flow and B) salinity.

3.4.2 Methodological approaches

Across studies, a variety of approaches were used to treat both trait data tables and to associate traits with flow or salinity. Concerning the flow-trait relationships, four of the 11 studies treated their trait tables by weighting each trait category with the abundance of their respective taxa (MM: Matrix multiplication), while the remaining seven papers used a number of different approaches including converting trait tables into Perc (percentage of taxon within each trait category), D (density of taxon possessing each trait for each sample), R (relative abundance of each taxon possessing each trait for each category), P (proportion of each taxon possessing each trait for each category), RF (relative frequency of each trait category), CV (coefficient of variation, calculated as the ratio of the standard deviation of each trait category to the mean); SPEAR [species at risk (based on the calculation of the fraction of the abundance of sensitive individuals in a community for a specific stressor)] (Table 2). There were similar variations in studies of salinity-trait relationships: three of the seven studies treated their trait tables by weighting each trait category with the abundance of their respective taxa, while the remaining four studies used a number of different approaches (Table 2).

Three studies used the fourth-corner approach (Dray et al., 2014) to link trait categories to flow, while eight used a number of different approaches (Table 2). There were similar variations in studies of salinity-trait relationships: three of the seven studies used ordination techniques to explore relationships between trait categories and salinity, while the remaining four used a number of different approaches (Table 2).

There were considerable variations in the numbers of traits used in both flow and salinity studies. Regarding flow-trait relationships, four studies used traits describing eleven biological characteristics, while the remaining seven used varying numbers of traits (Table 2). Five of the eleven studies used mixed level of taxonomic resolution while the remaining six used only one level (either family or genus) (Table 2). There were variable taxonomic scope among flow-trait studies. Six papers used traits information for most major macroinvertebrates groups (insects, including chironomids, crustaceans, mollusks, and oligochaetes) but excluded mites, while the remaining five studies either included mites with the aforementioned groups or excluded Oligochaeta, chironomids and mites from their trait information (Table 2). There were similar variations in studies of salinity-trait relationships: three used traits describing eleven biological characteristics, while the remaining eight used

varying number of traits (Table 2). Five papers used mixed level of taxonomic resolution in their traits information, while the two studies used only genera (Table 2). There was variable taxonomic scope among salinity-trait studies. Four studies used trait information for all macroinvertebrates excluding mites for their studies.

Metrics that described ecologically relevant aspects of the magnitude and frequency of flow (Allan et al., 1997) were used. Four of the seven flow-traits studies used flow permanence while the remaining seven used other metrics. All salinity-trait studies used electrical conductivity as a proxy for salinity, but none investigated different ionic concentrations. All the studies (flow and salinity) were correlative survey except one, which was based on an experiment.

Table 2. Summary of methodological approaches among flow-trait and salinity-trait studies**.** Abbreviations in the columns represent the following: taxonomic scope; Exc (excluding), Macro [macroinvertebrates (includes insects, crustaceans, mollusks, chironomids (chiro), worms (oligochaetes) and mites], flow/ salinity metrics; FlowPerm (flow permanence), FlowDur (flow duration), FlowConnec (flow connectivity), DATSam (discharge at time of sampling), EC (electrical conductivity, k_{25}, a proxy for salinity), trait conversion; Perc (percentage of taxon within each trait category), D (density of taxon possessing each trait for each sample), R (relative abundance of each taxon possessing each trait for each category), P (proportion of each taxon possessing each trait for each category), RF (relative frequency of each trait category), MM (Matrix multiplication: weighting each trait category with the abundance of their respective taxa), CV (coefficient of variation), SPEAR [species at risk (based on the calculation of the fraction of the abundance of sensitive individuals in a community for a specific stressor)], analysis; RSR (relative species retention), and RDA (redundancy analysis)

References	Taxonomic scope	Flow/ salinity metric	Taxa resolution	Number of biological traits	Trait conversion	Analysis (linking traits to environmental variables)	Source of trait information
Flow (N = 11)							
Arscott et al. (2010)	Macro	FlowPerm, FlowDur	Family, Genus, Tribe, Order	4	Perc	logistic regression	Greenwood & Booker, 2015
Brooks and Haeusler (2016)	Macro (Exc. Mites)	FlowVel	Genus	2	D	linear quantile regression	Poff et al. (2006); Schäfer et al. (2011)
Cid et al. (2016)	Macro	FlowConnec	Family, Order	11	R	traits metrics related to flow	Tachet et al. (2000)
Chessman (2015)	Macro (Exc. Mites)	DATSam	Family	7	P	Spearman's correlation	Schäfer et al. (2011)
Brooks et al. (2011)	Macro (Exc. Mites)	FlowPerm	Family, Tribe	4	Standardized trait data by dividing each variable value by the maximum value for that variable	separate ordinations for families, traits and environmental variables	Poff et al. (2006)
Walters (2011)	Macro (Ex. Mites)	DATSam	Family	6	R, MM	linear mixed effect model	Poff et al. (2006)
Bêche and Resh (2007)	Macro (Ex. Mites)	DATSam	Genus, Family, Order	16	MM, mean, standard and CV at each site for each year	Spearman's correlation	Bêche & Resh, 2007
Bêche et al. (2006)	Macro (Ex. Chiro,	DATSam	Genus	16	P, MM	ordinations,	Resh et al. (1994),

	worms and mites)					Spearman's correlation	Tachet et al. (2000); Usseglio-Polatera et al. (2000), Beche (2005)
Gallardo et al. (2009)	Macro (Ex. Chiro, worms and mites)	FlowConnec	Genus	11	none	Fourth-corner	Tachet et al. (2000)
Garcia-Roger et al. (2013)	Macro (Ex. Mites)	FlowPerm	Family, Order	11	RF, MM	Fourth-corner, Rao's diversity for each trait and sample	Tachet et al. (2000); Usseglio-Polatera et al. (2000)
Bonada et al. (2007)	Macro (Ex. chiro, worms and mites)	FlowPerm	Genus	11	none	fourth-corner	Usseglio-Polatera et al. (2000)
Salinity (N = 7)							
Gallardo et al. (2009)	Macro (Ex. chiro, worms and mites)	EC	Genus	11	none	Fourth-corner	Tachet et al. (2000)
Szöcs et al. (2014)	Macro (Ex. Mites)	EC	Genus	21	RF, MM	Ordination using RDA	Usseglio-Polatera et al. (2000)
Piscart et al. (2006)	Macro	EC	Genus, Order	8	RF, MM	Variance for each trait	Usseglio-Polatera et al. (2000)
Díaz et al. (2008)	Macro	EC	Genus, Family, Tribe	11	none	RLQ analysis	Tachet et al. (2000)
Kefford et al. (2012)	Macro (Ex. Mites)	EC	Family, Species	8	Mean value of each trait modality	RSR	Schäfer et al. (2011)
Vidal-Abarca et al. (2013)	Macro (Ex. Mites)	EC	Genus, Species	11	RF, MM	ordinations	Tachet et al. (2000), Bonada & Dolédec (2011); Picazo et al. (2012)
Schäfer et al. (2011)	Macro (Ex. Mites)	EC	Family, Genus	9	SPEAR	Spearman's correlation	Schäfer et al. (2011)

3.4.3 Trait responses to flow and salinity across studies

During high flows, studies generally reported an increase in traits that described morphological and ecological characteristics of macroinvertebrates, rather than in other trait groups (e.g. life history and mobility). The prevalence of no body armouring (not sclerotized), large body size, flattened body shape and filter feeders increased during high flow events while aerial dispersal and aerial respiration decreased (Table 3). None of the high flow events documented were floods (i.e. over-bank flows).

During low flow events, studies generally reported an increase in traits that described life history, mobility, morphology and ecology, as well as a decrease in morphology and ecology trait groups (Table 3). The prevalence of filter feeders and high rheophily decreased while the following all increased: desiccation resistance, aerial dispersal, aerial respiration (via spiracles), small body size, heavily sclerotized bodies, high thermophily, swimmers and fliers (Table 3). Inconsistent traits found during low flow events were small body size, shredders, tegument respiration and burrowers. Three of seven studies that used small body size found it to increase during low flows (e.g. Bêche et al., 2006, Bêche and Resh, 2007, Garcia-Roger et al., 2013) while only one reported a decrease (Walters, 2011). Two of six studies that used shredders found a decrease during low flows (Bêche and Resh, 2007, Brooks et al., 2011) while one study (Bonada et al., 2007) reported an increase (Table 3). Two of the six studies that used tegument respiration found an increase during low flows (e.g. Brooks et al., 2011, Brooks and Haeusler, 2016), while another two (e.g. Bêche and Resh, 2007, Bonada et al., 2007) reported a decrease. Two of the six papers that used burrowers found an increase during low flows (e.g. Bêche and Resh, 2007, Walters, 2011), while only one study (e.g. Bonada et al., 2007) reported a decrease. Only one paper examined trait responses to low flows as a result of drought. Fast maturation and aerial respiration increased while slower maturation, high rheophily and low thermophily decreased during drought.

At high salinities, trait groups that described life history, mobility, morphology and ecology generally increased (Table 3). The prevalence of ovoviviparity, multivoltinism, aerial dispersal, strong swimming ability, aerial respiration, heavily sclerotized, predators and swimmers increased during high salinity, while gill respiration decreased. The only inconsistent trait found at high salinities was shredders. One study found an increase (Szöcs et al., 2014) in the prevalence of shredders while another reported a decrease (Kefford et al., 2012) (Table 3). Only one study examined trait responses to low salinities. During low

salinities, univoltinism, oviposition in clutches and tegumental respiration increased in prevalence while aerial respiration decreased as salinity in this range increased (Table 3).

Table 3. Summary of macroinvertebrate traits response to flow and salinity. Different studies covered different ranges of salinity (see Appendix 2 for the range of salinity used for individual studies). Equivocal traits here indicate those traits have been found to increase by some authors while other authors have reported a decrease for the same type of environmental feature (e.g. high or low flows, and high or low salinities). The numbers in bracket (e.g. 1/3) represent the number of studies that found an increase or decrease for a particularly trait out of the total number of studies that used that particular trait in analyses. The symbols in brackets (e.g. +++, -/ 7) means 3 out of 7 studies reported an increase for that particular trait while one reported a decrease.

Environmental variable/ feature	**Traits**		
	Increase	**Decrease**	**Equivocal**
	Flow (N = 11)		
High flow (e.g. flow duration, permanence or connectivity)	**Life history** Long life cycle (1/3); univoltinism (1/3); holometaboly (1/1); egg (aquatic stage) (1/5); isolated eggs (parental care) (1/4); fixed, single eggs (egg type) (1/4) **Mobility** attached eggs (1/4); **Morphology** no body armouring (not sclerotized) (2/3); Large body size (2/7); flattened body shape (2/2) **Ecology** Filter feeders (3/4); detritus feeders (1/3)	**Life history** Larvae (aquatic stage) (1/5); endophytic (parental care) (1/3) **Mobility** Aerial dispersal (2/3); **Morphology** heavily sclerotized (1/3); aerial respiration (2/4)	
High flow as a result of flood	No studies	No studies	
Low flow (e.g. flow duration, permanence or connectivity)	**Life history** Desiccation resistance (5/7); larvae (1/5); endophytic (parental care) (1/3); asexual reproduction (1/3) **Mobility** High crawling rate (1/1); aerial dispersal (4/5); **Morphology**	**Life history** Adult ability to exit water (1/1) **Morphology** No sclerotized (soft bodied) (1/4);; Gill respiration (1/7) **Ecology**	**Morphology** Small body size (+++, -/7) Tegument/ cutaneous respiration (++, - -/6) **Ecology** Shredder (detritivore) (- -, +/6) Burrow (++, -/6)

	aerial respiration (via spiracles) (3/5); heavily sclerotized (3/4); **Ecology** High thermophily (2/2); swimmers (3/5); fliers (3/5); feeding on dead animals (1/3)	Filter feeders (2/6); high rheophily (2/2); clinging preferences (1/2);	
Low flow as a result of drought	**Life history** Faster maturation (1/1) **Morphology** Respiration via spiracles (aerial respiration) (1/1)	**Life history** Slower maturation (1/1) **Ecology** High rheophily (1/1); low thermophily (1/1)	
Salinity (N = 7)			
High salinity 4,000 µS/cm ≤ salinity ≤ 130,084 µS/cm	**Life history** Ovoviviparity (4/7); Multivoltinism (4/7); asexual reproduction (1/4); low physiological sensitivity to salinity (1/1) **Mobility** ability to drift (1/2); aerial dispersal (3/5); strong swimming ability (2/4) **Morphology** aerial respiration (3/6), heavily sclerotized (3/4) **Ecology** Parasites (1/3); Predators (3/3); swimmers (2/4); Tegument respiration (1/4)	**Life history** Egg (aquatic stage) (2/6); high physiological sensitivity to salinity (1/1) **Morphology** Gill respiration (2/6)	**Ecology** Shredders (+, -/6)
Low salinity 0µS/cm < salinity < 4,000 µS/cm	**Life history** Univoltinism (1/1); ovipositing in clutches (1/1) **Morphology** Tegument respiration (1/1)	**Morphology** Aerial respiration (1/1)	

3.5 Discussion

The results of this review showed that the number of publications on macroinvertebrate traits response to effects of flow and salinity are accumulating at a slow, but steady rate, but the small sample sizes and divergent methods used preclude any formal or meaningful meta-analysis at this stage. The majority of these flow-trait studies have been conducted in Europe and Oceania, while salinity-trait studies have been based predominantly in Europe. This emphasizes the need for more research in underrepresented regions such as Asia, Africa and South America. The results also show heterogeneity of responses of traits to effects of flow and salinity, potentially partly due to differences in methodological approaches among studies.

3.5.1 Temporal and geographic scope

The steady but slow accumulation of flow-trait and salinity-trait publications highlights the need for more studies. These two stressors are globally held to be major drivers of aquatic communities, especially in dry environments (Williams, 2002). The geographic bias in research also hinders efforts to generalise about how salinity and flow affect macroinvertebrate trait composition. Given the projections of increased dry periods in many of the world's major river basins owing to climate change (Prudhomme et al., 2014), the demand for water, globally, is predicted to increase (Van Huijgevoort et al., 2014). Consequently, more research is needed urgently on flow-trait and salinity-trait relationships in under-represented regions to establish baseline information that may help improve the diagnostic ability of bioassessment based on traits (Culp et al., 2011, Van den Brink et al., 2011).

Our review also showed that flow-trait studies were dominated by surveys conducted across multiple catchments; however, studies at spatial longitudinal scales are also necessary to more fully understand effects of flow and salinity. For example, Richards et al. (1997) found that reach-scale physical features (e.g. habitat parameters at each site, substrate characteristics, bank conditions, hydraulic characteristics) predicted life history and behavioural traits of macroinvertebrates better than catchment-scale variables, suggesting that traits exhibit strong relationships with local environmental conditions.

3.5.2 Methodological approaches among studies

Our review showed that several methodological approaches have been used to associate traits to flow or salinity, as well as explore patterns in trait-based community studies. It is worth noting that the outcome of trait-based community patterns may be influenced by the taxonomic resolution of the taxa characterised by traits (family, genus, species or mixed), the number and type of traits being considered, trait data conversion (quality) and how trait-environment relationships are quantified (Heino et al., 2013). Unfortunately, the sample sizes realised in this review are too small to make definitive recommendations on preferred methodological approaches and authors should be encouraged to make raw data sets openly accessible to encourage comparisons between the methodological approaches.

We found that trait data tables usually showed a mixed resolution of taxonomic levels, especially for more cryptic taxonomic groups, such as the Chironomids and Oligochaetes. Although these taxonomic groups are important in quantifying both diversity patterns and ecosystem processes in a variety of habitat types, logistical constraints and the need for high levels of taxonomic expertise hinders identifications to levels lower than order or family for these often abundant groups, such as Acaria, Oligochaeta and Chironomidae (Franquet, 1999). Therefore, studies that are unable to identify these to lower taxonomic levels either use coarser taxonomy (e.g. Cid et al., 2016) or exclude them from the analysis (e.g. Gallardo et al., 2009). Omitting such taxa could restrict the suite of trait categories available for identifying the type and level of environmental impact (Culp et al., 2011), while assigning traits at coarse taxonomic resolution may obscure important responses as demonstrated by Serra et al. (2016). Furthermore, deficiencies in knowledge of trait values to accord some macroinvertebrate taxa is problematic for comparability. For instance, in our review, some studies contained only genus-level data for a limited number of traits (e.g. Brooks and Haeusler, 2016) (Table 1) or presented numerous traits for taxa with mixed levels of taxonomic resolution (e.g. Bêche and Resh, 2007). The ideal scenario may be to use the same trait database (at least, as long as trait allocation is conducted at coarse scheme of classifying macroinvertebrates, rather than say, detailed measurement of individual body sizes, for example. Such coarse scheme of allocating traits may reasonably be applied to biota from different regions as long as genus and species names are the same) across studies, resolved at genus or species level as these levels have the greatest potential to improve the signals

provided by ecological assessment tools (Serra et al., 2016). This will help make inter-study comparisons more straightforward (Statzner and Bêche, 2010).

The choice of traits may also influence comparison of studies among different regions or different habitat types (Petchey and Gaston, 2006). For instance, if a study focusses on ecosystem processes, the choice of traits evaluated may be explicitly related to the specific process (Brooks and Haeusler, 2016). By contrast, if the interest is in trade-offs among traits, then the whole set of traits would be more appropriate (Poff et al., 2006). An example of a study where specific traits could have been chosen is Szöcs et al. (2014), where in determining the effect of salinity on biological traits, used the whole set of twenty-one traits from Usseglio-Polatera et al. (2000a), rather than only those traits hypothesized to be related to salinity.

Additionally, traits may often be phylogenetically linked (trait "syndromes") (Bêche and Resh, 2007, Poff et al., 2006) and may respond similarly to an environmental gradient, creating redundancies that may complicate interpretation (Poff et al., 2006, Poteat et al., 2015). For example, Van Kleef et al. (2006) found that ability to fly was not important in the re-colonisation of restored habitats. This counterintuitive result arose because many species with active flight in their study were also carnivorous, and hence recruitment was delayed by scarcity of prey. As a possible solution to the potential problem of trait syndromes, Poff et al. (2006) suggested using phylogenetically independent traits such as thermophily, rheophily, crawling abilities, among others. Furthermore, the challenges associated with trait syndromes may be minimized when only traits that are hypothesized to have mechanistic links with stressors of interest are chosen (Culp et al., 2011).

Even after one has selected traits relevant to a research question, the question of how these traits can be quantified for characterising ecological communities is challenging. For instance, trait tables may be expressed on different measurement scales (e.g. nominal, ordinal, interval, ratio or quantitative variables) (Schmera et al., 2015). Ordinal and mixed data sets pose challenges for similarity-based analysis (Pavoine et al., 2009, Schmera et al., 2015), some of which can be overcome by using appropriate similarity measures for ordinal data such as Gower's similarity index (Anderson et al., 2006, Gower, 1971). However, these recommendations are sometimes not followed [e.g. Brooks et al. (2011), who analysed ordinal data using Euclidean metric]. This diversity of methods is a major impediment to synthesis.

Consequently, the summarised output from trait-based analysis might be one of a number of different forms: a multivariate point pattern (e.g. Bêche et al., 2006) or a measure of variance (e.g. Bêche and Resh, 2007), and these measures of trait variation may be based on either abundance (e.g. Walters, 2011) or presence-absence data (e.g. Arscott et al., 2010). However, Schmera and Erős (2011) in comparing caddisflies populations from two different samples demonstrated that the abundance of taxa and abundance-weighted traits contributes to a better separation of communities in relation to environmental variation.

3.5.3 Trait responses to flow and salinity across studies

Generally, high flows favoured invertebrates with no body armouring (not sclerotized), large body size, flattened body shape and filter feeders, while aerial dispersal and aerial respiration decreased. In contrast, low flows promoted aerial dispersal, desiccation resistance, aerial respiration, small body size, heavily sclerotized, high thermophily, swimmers and fliers, while filter feeders and high rheophily decreased.

The ecological explanations supporting these trait relationships with high and low flows are as follows. High flows are characterised by high concentrations of dissolved oxygen and lower temperatures, and the faster water currents offer advantage for filter feeders to take advantage to feed by filtering food particles carried by the flowing stream (Brooks and Haeusler, 2016, Cid et al., 2016). In contrast, low flows are characterised by low dissolved oxygen and higher temperatures, and these conditions may offer advantage for macroinvertebrates that are desiccation tolerant (Walters, 2011), have morphological structures to breathe atmospheric air (Bonada et al., 2007), are tolerant of higher temperatures (Chessman, 2015), and have the ability to move as habitat contracts (e.g. fliers, or taxa which have strong swimming abilities) (Chessman, 2015, Horrigan and Baird, 2008).

Shredders, small body size, tegument respiration and burrowers were involved in inconsistent responses to low flows. Low flows may lead to declines in shredder population due to a reduction in quantity of leaf litter that falls into streams owing to the effect on riparian vegetation (Cummins et al., 1989) and the unfavourable conditions that characterize low flows (Tomanova et al., 2008). Furthermore, small body size is viewed as an adaptation which offers better resilience to low flows because small-sized macroinvertebrates may be able to find new refuges during drying up of streams, owing to their ability to be dispersed by wind easier compared to large-sized macroinvertebrates (Arscott et al., 2010). Lastly, the

ability to burrow has been linked to desiccation resistance for some aquatic insects in intermittent streams (Acosta and Perry, 2001, Boulton and Stanley, 1995) during low flows. However, an increase in burrowers have been found in streams with high flows and low salinities (C. Madden, *pers comm.*), suggesting that burrowing may not only be an adaptation to survive desiccation but also an adaptation to withstand being washed away by faster water during high flows.

Further, the inconsistencies in traits response to effects of flow and salinity we observed cause us to suspect that there might have been interactions between salinity and flow, occurring separately in those studies [e.g. Walters (2011) and Bonada et al. (2007)] which might have resulted in some traits (e.g. burrowers and tegument respiration) responding differently to low flows. For example, Walters (2011) found that a low flow disturbance in Connecticut, North America promoted burrowers, while Bonada et al. (2007) reported the opposite response during an observational study of traits response to low flows in streams in Catalonia, Spain. However, the stream sites used in these studies were located in catchments that were also affected by natural salinization. Interestingly, these studies did not examine interactions between salinity and flow. Therefore, further studies is needed to examine whether interactions between flow and salinity underlie these inconsistencies in trait literature. Such studies will provide insight as to whether variations among studies may also be due to underlying ecological reasons, and not only due to differences in methodological and analytical techniques.

Generally, high salinity promoted ovoviviparity, multivoltinism, aerial respiration, heavily sclerotized, predators and swimmers, while egg (aquatic stage) and gill respiration decreased. Low salinity promoted univoltinism, tegument respiration, oviposition in clutches while aerial respiration decreased, although this finding was in only one study. Ovoviviparity is favoured because it protects the young by isolating the eggs from external elevated salt concentrations (Piscart et al., 2006). Multivoltine life cycles are held to be advantageous since it increases the chances of survival of offspring (Kefford et al., 2004). Young individuals commonly have a lower salinity tolerance than older stages (Kefford et al., 2004), and are likely to grow slowly in response to flow pulses of high saline water, thus making adult females produce more than two generations per year to increase chances of survival of their offspring. Predators (e.g. most coleopterans) are favoured in saline streams because they

may possess more energy for osmoregulation (Abellán et al., 2007) and are able to hunt prey which are not well suited for saline streams.

3.6 Conclusion

Trait-based analysis provide a key tool to understanding variation in community structure in streams. While there are some broad qualitative trends in which traits appear to be correlated with flow and salinity, further quantitative conclusions are impeded by the following issues: (1) the divergent methods used to translate tables of taxa by traits into input data for statistical analyses, as indicated by Monaghan and Soares (2014) and Schmera et al. (2014); (2) variation in taxonomic resolution and the level of resolution used in assigning trait categories. If further studies reveal that assigning traits at coarse taxonomic resolution for numerous groups (e.g. Chironomidae) results in biases or unreliable interpretations as demonstrated by Serra et al. (2016), then the extra resources needed to resolve taxonomy more finely will lessen any cost-savings resulting from using traits instead of taxonomy; (3) the variation of methods used to relate input data to flow and salinity variables is a major impediment to synthesis; (4) the lack of standardisation of trait classifications, as indicated by Schmera et al. (2015), makes it difficult to translate information from one study to the broader ecological literature. To foster the operative use of species traits in the field of stream ecology, there is the need for consolidated and comprehensive trait databases resolved at genus or species level, standardized in terms of trait classifications and nomenclature, with more definitive recommendations on preferred methodological approaches.

3.7 Supporting information

Table S1. Keywords used in my search for literature on trait responses to effects of flow and salinity

Table S2. Summary of flow-trait and salinity-trait studies used in our review. Table shows the range of salinity values covered for individual studies. Abbreviation in scale of study column represent; L: longitudinal scale; MC: multiple catchment; and C: catchment scale

Chapter 4

Effects of salinity and flow interactions on macroinvertebrate traits in temporary streams

The Mawson Plateau located in the Flinders Ranges in South Australia (Photo by Stephen Reynolds)

Chapter 4 is in review in *Ecological Indicators*:

Botwe, P. K., Carver, S., Magierowski, R., McEvoy, P., Goonan, P., Madden, C., Barmuta, L. A. Effects of salinity and flow interactions on macroinvertebrate traits in temporary streams

4.1 Summary

Increasing salinity in freshwater ecosystems is globally widespread, especially, in arid and semi-arid regions, and can co-occur with flow intermittency, particularly in temporary streams. Both these stressors are known to affect macroinvertebrate traits individually, but their interactive effects have not been previously considered. There are inconsistencies reported in literature regarding the response of particular traits to flow or salinity, and accordingly, we hypothesized that interactive effects between these two stressors may underlie inconsistencies in the literature. We used multivariate and univariate approaches to investigate the effects of salinity and flow interactions on macroinvertebrate traits using 13 years of data sampled across multiple sites in South Australia, the driest state in the driest inhabited continent in the world. Ovoviviparity, multivoltinism, aerial respiration and strong fliers were favoured as salinity increased, while medium-high physiological sensitivity to salinity and respiration via gills decreased. During low flows, holometaboly, univoltinism, high rheophily, cool eurythermal, streamlined body shape and gill respiration decreased, while aerial respiration and fliers and high crawling rate increased. Interestingly, traits with inconsistent behaviour in the literature were associated with interactions between flow and salinity. Burrowing, tegument respiration and collector-gathering were associated with interactions between low flows and salinity. These traits showed a similar interaction, with all traits being least abundant in streams with high salinity and low flows, and low salinity and high flows. The interactions seem to be driven by the differential response of different taxa with the same trait category being abundant in different parts of the space defined by the interaction plots. Our findings suggest that, in addition to differences in methodological and analytical approaches, interactions may also underlie inconsistencies in trait responses to flow and salinity. Finally, to foster the operative use of traits to resolve the effects of multiple stressors on ecosystems, there is the need for the development of a better mechanistic understanding of how specific stressors (e.g. flow and salinity) act as trait filters, potentially through the use of experiments, to ensure that each of the stressors is strong enough to produce clear trait responses.

4.2 Introduction

Flow intermittency and salinity characterise temporary streams and, when studied separately, are held to be major drivers of aquatic communities in dry environments (Williams, 2002). Surprisingly, few studies address the combined or interactive effects of these stressors despite

their known, separate effects on biodiversity and functioning of aquatic ecosystems (Moreno et al., 2010), including nutrients and water cycling (Arscott et al., 2010, Herbert et al., 2015). Furthermore, the generality of applying the results of many flow and salinity studies on aquatic invertebrates to other regions is limited by the biogeographic variability in taxonomic composition (McGill et al., 2006). Thus, comparable approaches, incorporating flow and salinity simultaneously, across study regions are essential to understand the effects of salinity and flow. In this study, we address this critical knowledge gap by tackling the individual and interactive effects of salinity and flow on macroinvertebrate traits across a large spatio-temporal scale in South Australia.

The effect of increased salinity (Schäfer et al., 2011) and flow intermittency (Palmer et al., 2015) have traditionally been assessed using taxonomically-based methods (macroinvertebrate identities, abundance and distribution), but comparisons and generalisations are difficult because different taxa occupy similar ecological niches in different biogeographic regions (Poff et al., 2006). Furthermore, taxonomically-based methods are often limited in their ability to distinguish mechanisms of impact (Townsend et al., 1997) and thus, the importance of different stressors or effects of multiple stressors (Wooster et al., 2012). In contrast, biological traits can provide a mechanistic understanding of stressor impacts (McGill et al., 2006), and therefore have the potential to identify the importance of different co-occurring stressors that influence different aspects of the environment (Statzner and Bêche, 2010). Therefore, macroinvertebrate traits have been proposed as an alternative approach to use (McGill et al., 2006).This potential for traits to be for traits to be used to identify the effects of multiple stressors (e.g. flow and salinity) on freshwater ecosystems may be useful because managers of these systems typically have multiple restoration options, and need decision support tools to make well-informed decisions about budget allocations for particular restoration measures (Niemi and McDonald, 2004)

To date, many studies have examined relationships between flow and macroinvertebrate traits (e.g. Bêche and Resh, 2007, Chessman, 2015, Walters, 2011), but fewer have examined relationships with salinity (e.g. Díaz et al., 2008, Szöcs et al., 2014). For flow, the most commonly documented patterns are that high flows favour traits such as slower maturation, high rheophily, low thermophily, holometaboly, lack of body armouring (not sclerotized) and filter feeding (e.g. Bêche and Resh, 2007, Chessman, 2015, Walters, 2011). Increased salinity promotes predators, multivoltinism, aerial respiration, aerial

dispersal and ovoviviparity (e.g. Díaz et al., 2008, Szöcs et al., 2014). However, some traits are equivocal or show inconsistent behaviour between studies. For instance, during low flows, Brooks et al. (2011) found an increase in the prevalence of tegument (cutaneous) respiring taxa, while Bonada et al. (2007) reported the opposite response. Similarly, Walters (2011) found that low flows promoted burrowers, while Bonada et al. (2007) found fewer burrowers under low flows. During increased salinity, Vidal-Abarca et al. (2013) found an increase in the prevalence of tegument respiration while Szöcs et al. (2014) reported the opposite response. It is possible that the inconsistencies observed in the trait literature may reflect interactions between flow and salinity as these two stressors are commonly linked. For example, the major environmental impact of flow intermittency can include decreased flow permanence, increased sediment deposition, increased water temperatures, low dissolved oxygen and loss of some habitats (Dewson et al., 2007), while impacts of salinization include increased temperature, low dissolved oxygen and loss of riparian habitats (Schroder et al., 2015). Thus, these two type of stressors have similar environmental impacts: both are expected to increase water temperature, decrease habitat complexity and reduce dissolve oxygen content, which suggests that, their effects could potentially interact none additively. Importantly, there have been no studies focussing on the relationships of both salinity and flow to traits, especially across multiple catchments.

One of the reasons for a paucity of studies simultaneously addressing effects of salinity and flow on invertebrate traits is that flow volume is often inversely correlated with salinity (Brock et al., 2005). To fully understand the combined effect of salinity and flow on macroinvertebrate traits, it is therefore necessary to study a full range of both factors. Such information is critical for identifying how both factors shape the structure and functioning of aquatic communities in dry environments.

Our study area in southern South Australia constitutes a gradient from Mediterranean through to arid, warm temperate climates, with flow conditions ranging from permanent to ephemeral. Temporary streams are abundant (Laut et al., 1977) and include a variety of combinations of flow and salinity. Aquatic invertebrates in this region have been exceptionally well sampled (13 sites, sampled bi-annually for 13 years) and thus present an ideal data set to test the response of traits to salinity and flow. Here we aim to identify the effects of salinity and flow interactions on the trait structure of macroinvertebrate communities.

4.3 Methods

4.3.1 Study area and macroinvertebrates sampling

Our study sites were distributed throughout Kangaroo Island (1 site from Rocky River), Yorke and Fleurieu Peninsulas (2 sites including Hill River and Kanyaka Creek), the Mount Lofty Ranges (MLR) [Western MLR = 7 sites including Hindmarsh, Torrens, North Para, Myponga and Light rivers, First and Scott creeks] and the lower Mid-North of South Australia (3 sites including Finniss, Marne and Bremer rivers) [Fig. S1 in Supporting Information]. Flows in these streams are largely driven by ground water which has accumulated marine-derived salts over long time-scales (Herczeg et al., 2001). In places, salinity is further exacerbated by clearing of native vegetation and irrigation for agriculture. There are no secondary sources of salinization such as from mining, coastal inundation, industrial discharge or road de-icing, and electrical conductivity is dominated by Na^+ (median: 72 % of cations) and Cl^- (median: 98% of anions), with similar ionic compositions across this region (Supporting Information Table S1).

The macroinvertebrate samples used in our analysis form part of the Australian Rivers Assessment System (AusRivAS) (Davies, 2000) of which South Australia has been part since 1994. The database includes a substantial, standardised record of benthic macroinvertebrates and a large number of environmental variables. Annual sampling was conducted in two seasons (autumn and spring) to avoid dried-out summer periods and low macroinvertebrate activity during winter. We used data collected for 13 years from 1994 to 2007 (excluding 1996 owing to a hiatus in funding).

Macroinvertebrates were collected using standardised AusRivAS protocols which consisted of sampling approximately 5 m^2 area of edge and pool habitats within each 100 m study site using a 250 µm mesh triangular dip net. Sampling involved vigorously kicking the substrate and sweeping the net over a total bank length of 10 m using sequential short sweeping movements at right angles to the bank and, sweeping under overhanging or emergent vegetation (Davies, 2000). Collected macroinvertebrates were preserved in ethanol on site, transported to the laboratory, and subsampled (where a minimum of 10% of the sample was counted and identified using dissecting and compound microscopes), and the residue scanned for rare taxa (Davies, 2000, Simpson and Norris, 2000). This approach ensured observer bias was minimised when counting individuals compared to alternative live-

pick approaches included in the AusRivAS protocols, and it also provided an accurate estimation of the abundance of cryptic taxa. Taxa were identified to the lowest taxonomic level, given available keys, life-history stage and condition. This was most often to genus or species level. Voucher specimens of all taxa were retained as a reference collection at the South Australia Museum and Australian Water Quality Centre (AWQC).

4.3.2 Traits

We used 75 biological traits grouped into four categories (life history, mobility, ecology and morphology) to describe the functional composition of invertebrate communities (Supporting Information Table S2). Trait values were assigned at family level (except for the Chironomidae, where traits were assigned at subfamily level) using existing trait databases of Poff et al. (2006) and Schäfer et al. (2011), and, where South Australian taxa were not covered in these sources, we utilised expert opinion from taxonomists and information from *Identification and Ecology of Australian Freshwater Invertebrates* (http://www.mdfrc.org.au/bugguide/, accessed January 2016). The trait databases from Poff et al. (2006) and Schäfer et al. (2011) described the affinity of each family to each category with scores ranging from "0" (no affinity) to "5" (high affinity) using the fuzzy coding approach of Chevenet et al. (1994). Schäfer et al. (2011) trait database is predominantly an Australian database (with most traits compiled from the Australian literature and expert opinion) with focus on detecting effects of pesticides and salinity. However, Poff et al. (2006) trait database is a North American database with a strong focus on detecting effects of flow alterations. We therefore selected only those traits that are hypothesized to be related to salinity and flow by Schäfer et al. (2011) and Poff et al. (2006) respectively, to allocate trait affinity scores to our Australian fauna. We used family level taxonomic resolution (except for Chironomidae, where subfamilies were used) because Gayraud et al. (2003) argued that the functional structure of communities is conserved if taxonomic levels higher than species are used, especially when analysed with multivariate methods.

Phylogenetically linked traits (trait "syndromes") (Bêche and Resh, 2007, Poff et al., 2006) may respond similarly or in tandem to an environmental gradient, creating redundancies that may complicate interpretation (Poff et al., 2006, Poteat et al., 2015). To minimise the impact of these issues, we followed Poff et al. (2006) and Culp et al. (2011) by choosing traits that were highly labile evolutionarily (e.g. rheophily, thermophily) and which

were also hypothesised as having mechanistic links with the environmental variables we were considering, i.e. flow and salinity (Chessman, 2015, Díaz et al., 2008).

Trait category scores were multiplied or weighted by the abundance of the taxa to obtain a sample-by-trait matrix that contains for each sample the averages of numerical traits over all macroinvertebrate taxa present (Walters, 2011).

4.3.3 Environmental and hydrological data

Salinity was measured *in situ* as electrical conductivity at 25 °C in µS cm^{-1} using calibrated water quality meters (Hach MP-6 portable meter). Work done by Williams (1966 and 1986) in South Australian lakes revealed a very strong relationship ($R^2 = 0.99$) between conductivity and salinity. Additionally, in South Australia, the most prominent influence of agriculture (and water abstraction) is to change stream flow regime, such that greater flow is derived from groundwater which flushes salts (predominantly Na^+ and Cl^- ions) from marine origin into streams (Boulton et al., 2014). Further, there are no secondary sources of salinization such as from mining, coastal inundation, industrial discharge or road de-icing, and electrical conductivity is dominated by Na+ (median: 72 % of cations) and Cl- (median: 98% of anions), with similar ionic compositions across this region. Therefore conductivity reflects or quantifies salinity alone. We hereby referred to conductivity as salinity.

All sites had flow-gauges from which mean daily flow data were compiled to calculate flow metrics. Six metrics that described ecologically relevant aspects of the magnitude and frequency of flow (Allan et al., 1997) were calculated in R version 3.2.4 (R Core Team, 2016) using base functions and those in the "hydrostats" package (Bond, 2015) (Table 1). These were: median flow (medQ) [magnitude], median cease-to-flow (med.CTF) [spell duration], annual average flow permanence (flowPerm) [magnitude] (Arscott et al., 2010), number of days since last low flow event 365 days prior to sampling (DSLE) [spell duration], number of days since the last high flow event (DSHE) 365 days prior to sampling [spell duration] and high flow disturbance (Q75_30) [magnitude] in the previous 30 days, which is classified as number of flow events ≥ 3 times the 75^{th} percentile discharge. We used three times the 75^{th} percentile discharge [modified from Greenwood and Booker (2015), because the median discharge of some of our streams was zero] as an indicator of high flow disturbance because flows exceeding this measure have been shown to reset invertebrate community structure (Arscott et al., 2010, Booker, 2013, Greenwood and Booker, 2015). The

magnitude of low flows was measured by the number of days since a low flow event (Table 1), and we defined a low flow event as the 80th percentile exceedence non-zero daily flow rate (Smakhtin, 2001, VanLaarhoven and Van der Wielen, 2009).

These flow variables were chosen because they captured independent aspects of flow duration and magnitude. We examined a wider range of flow variables initially, but after we examined co-linearity among normalized salinity and flow variables using Spearman's correlation coefficient (ρ) and scatter plot matrices, we selected variables from correlated sets that were likely most proximally related to the traits (Clarke and Warwick, 2001), in order to reduce redundancy. For example, we found that annual average flow permanence (flowPerm) was highly negatively correlated ($\rho = -0.75$) with median cease-to-flow (med.CTF), and flowPerm was used instead of med.CTF because we envisaged invertebrate traits responding to flow permanence rather than the median period of cessation of flow (Arscott et al., 2010) (Supporting Table S3).

The final selected flow variables were not strongly correlated with one another (all $\rho < |0.64|$), nor was salinity strongly correlated with any of these flow variables (all $\rho < |0.41|$, Supporting Information Table S3).

Table 1. Salinity and flow metrics calculated for the sites included in the analysis. Min: minimum value; Max: maximum value; SD: standard deviation.

Parameter	Abbreviation	Description	Units	Min	Max	Mean	SD
Environmental variable							
salinity	Cond	Electrical conductivity, K_{25}	µS cm^{-1}	139	23700	3709	4343
Hydrological regime							
Median flow	medQ	Median daily discharge on date of sampling	ML/ day	0.00	15.84	2.40	3.20
Median cease-to-flow	med.CTF	Median daily cease-to-flow discharge over 365 days prior to sampling	ML/ day	0.00	355	49.27	64.99
Annual average flow permanence	flowPerm	% time that flowing water was present over 365 days prior to sampling	%	0.00	100	78	26
Days since low flow event	DSLE	Number of days since there was a low flow event in 365 days prior to sampling	days	0.00	1202	119.2	164.93
Days since high flow event	DSHE	Number of days since there was a high flow event in 365 days prior to sampling	days	0.00	782	67	133.38
High flow disturbance	Q75_30	Number of flow events exceeding 3 × the 75th percentile daily discharge, 30 days prior to sampling	Count	0.00	81.41	8.33	12.02

4.3.4 Statistical analysis

4.3.4.1 Changes in trait composition

We explored changes in trait composition with three factors (site, season and year) using permutational multivariate analysis of variance (PERMANOVA) (Clarke and Warwick, 2001) based on a Gower dissimilarity (Anderson et al., 2006, Gower, 1971) of the log-transformed traits matrix. Site was treated as random with season and year as fixed factors, and formal testing was undertaken using the PERMANOVA+ (v1.0.6) software extension to PRIMER (v6.1.16) (Clarke and Gorley, 2006).

4.3.4.2 Relationship of macroinvertebrate trait composition to flow and salinity

To relate trait composition to the environmental variables, we used multivariate generalized linear mixed models (MGLMMs) with site as a random factor, to derive the most parsimonious models predicting trait composition using PERMANOVA+ with the predictor variables (flow metrics and salinity) fitted as fixed covariates. We used distance-based redundancy analysis (dbRDA) to visualize the relative contributions of each predictor to the trait structure (Legendre and Anderson, 1999, McArdle and Anderson, 2001).

4.3.4.3 Visualisation of trait responses

To examine which trait states were related to the significant predictors identified by the MGLMMs, we first used BVSTEP (Best Subset of Environmental Variables with maximum Correlation with Community Dissimilarities) in PRIMER (Clarke and Gorley, 2006, Clarke and Warwick, 2001) to find a subset of traits which were best correlated with patterns in the predictor variables (as represented by the distance matrix between predictors) and tested using 9999 permutations (Clarke and Ainsworth, 1993). Diagnostic analysis using variance inflation factors (VIF) were employed to check for multicollinearity (correlations between predictor variables): this was not an issue because all the VIFs were < 2.5 (Berk, 2003). Individual Spearman's rank correlations (ρ) were then used to evaluate the direction and strength of the relationships between each trait category and each predictor. Where there were significant interactions between hydrological variables and salinity, we used generalized additive mixed models (GAMMs) [using R version 3.2.4 (R Core Team, 2016)] with site as a random factor to generate heat maps to visualise the patterns in the interaction. These were

complemented by inspecting bubble plots of the abundance of the most abundant taxa within trait categories to understand how taxonomic patterns contributed to these interactions

4.4 Results

Overall, 274 samples were collected which comprised 130,383 individuals from 840 taxa. Salinity ranged from 139 – 23700 µS cm^{-1} and the ranges of values for the flow variables across sites were: median flow (0 – 15.84 ML/ day), median cease-to-flow (0 – 355 ML/ day), average flow permanence (0 – 1 %), days since low flow event (0 – 1202 days), days since last high flow event (0 – 782 days), high flow disturbance events (0 – 81.41) (Table 1).

4.4.1 Relationship of macroinvertebrate trait composition to flow and salinity

We evaluated seasons independently because the trait structure differed between autumn and spring [PERMANOVA test for Season; Pseudo $F_{(1, 88)} = 6.06$, $P = 0.007$]. Trait structure also differed between sites (Pseudo $F_{(12, 88)} = 9.83$, $P < 0.001$) and years (Pseudo $F_{(12, 88)} = 1.61$, $P = 0.027$), but we were explicitly interested in how flow and salinity shaped trait structure over time and we accounted for variation across sites by including site as a random effect in our models. In both seasons, there was significant random variation between sites. Salinity was significantly related to trait structure (MGLMM: Table 2), and samples within sites tended to cohere and remain separate in the dbRDA ordinations (Fig 1). In autumn, flow variables (DSLE: number of days since low flow event and flowPerm: flow permanence) were only significant if they were involved in interactions with salinity. Similarly, in spring, flow variables [Q75_30 (high flow disturbance) and flowPerm] were involved in interactions with salinity. Surprisingly, modest amounts of variation in the trait matrix was explained by the flow and salinity variables (27.3% in autumn, 36.7% in spring) (Table 2) relative to variance explained in taxonomic space (20.9% in autumn, 27.7% in spring).

Table 2. Results from multivariate generalized linear mixed models (MGLMM) for the 13 sites in autumn and spring. Bold numbers indicate significant *P*-values < 0.05. Site is treated as a random factor. Abbreviations for predictor variables are listed in Table 1 (Cond: salinity; Q75_30: high flow event > 75th percentile in the previous 30 days; flowPerm: flow permanence; DSLE: number of days since last low flow event; DSHE: number of days since last high flow event; and medQ: median flow).

Variable	***df***	***F***	***P***
Autumn (R^2 = 27.3 %; AICc = -407.96)			
Site	12	4.85	< **0.001**
Cond	1	1.53	**0.017**
Q75_30	1	0.80	0.522
flowPerm	1	1.53	0.183
DSLE	1	0.34	0.895
DSHE	1	1.36	0.215
medQ	1	1.66	0.148
Cond × DSLE	1	2.81	**0.040**
Cond × Q75_30	1	1.10	0.309
Cond × medQ	1	0.86	0.463
Cond × flowPerm	1	2.04	**0.029**
Spring (R^2 = 36.7 %; AICc = -415.29)			
Site	12	5.73	< **0.001**
Cond	1	1.78	**0.031**
Q75_30	1	2.73	**0.048**
flowPerm	1	0.43	0.785
DSLE	1	1.37	0.230
DSHE	1	0.80	0.481
medQ	1	0.89	0.412
Cond × DSLE	1	0.77	0.490
Cond × Q75_30	1	1.74	**0.015**
Cond × medQ	1	0.74	0.515
Cond × flowPerm	1	1.40	**0.041**

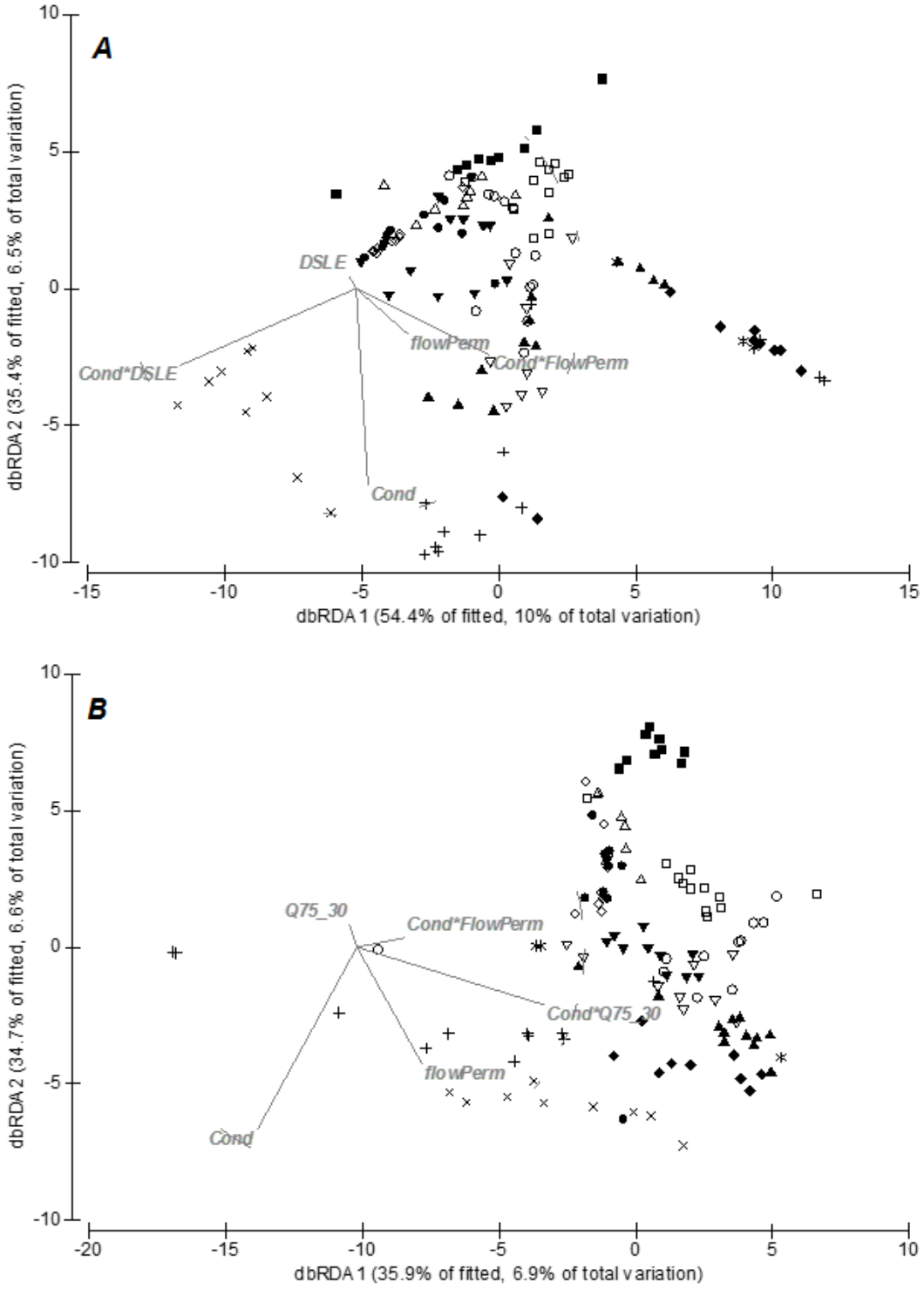


Fig 1. Distance-based redundancy analysis (dbRDA) of trait composition across years in (A) autumn and (B) spring, overlaid with normalised predictor variables (based on MGLMM analysis in Table 1). The straight lines (sites) observed in autumn is because autumn was relatively dry especially for the northern sites (have high salinity), therefore these sites were dominated by taxa that tolerate high salinity and low flow events. Abbreviations for predictor variables are listed in Table S3. Different symbols represent different sites (random effect) used in the study and their meaning are listed in Table S1 (▲: Bremer River; ▼: Finniss River; ■: First Creek; ♦: Hill River; ●: Hindmarsh River; +: Kanyaka Creek; ×: Light River; *: Marne River; Δ: Myponga River; ∇: North Para River; □: Rocky River; ◊: Scott Creek; ○: Torrens River).

4.4.2 Visualisation of trait responses

Nine of the 75 trait categories were significantly related to the strongest predictors of trait composition in autumn (BVSTEP: $P < 0.001$, $\rho = 0.41$) (Table 3; Table S4 in Supporting Information). As has been found elsewhere, ovoviviparity, strong flying strength (fliers) and respiration via plastron or spiracle (aerial respiration) were favoured in increased salinity, while gill respiration decreased. During low flows, high rheophily, cool eurythermality and respiration via gills decreased, while aerial respiration and fliers increased. As flow permanence increased, high rheophily and cool eurythermal were also favoured (Table 3).

During spring, eight trait categories were significantly related (result from BVSTEP: $P < 0.001$, $\rho = 0.50$) to the strongest predictors of trait composition (Table 3; Supporting Information Table S4). As has been found elsewhere, multivoltinism was favoured in higher salinity while medium-high physiological sensitivity to salinity decreased. During high flows, holometaboly, univoltinism and streamlined body shape were promoted while high crawling rate decreased. As the flow permanence increased, slow maturation increased (Table 3).

Interestingly, traits with inconsistent behaviour in the literature were associated with interactions between flow and salinity (Table 3). For example, burrowing, tegument respiration and collector-gathering were associated with interactions between number of days since low flow event (DSLE) and salinity in autumn. Burrower, collector-gathering and tegument respiring traits showed a similar interaction, with all traits being least abundant in streams with high salinity and shorter DSLE [extreme low flows or drier periods; darker region: bottom right of Figs. 2A, 2B and 2C] and low salinity and longer DSLE [wetter periods; darker region: top left of Figs. 2A, 2B and 2C].

Burrowing was also associated with an interaction between flow permanence (flowPerm) and salinity in spring, and, surprisingly, no single trait was significantly associated with the interaction between Q75_30 and salinity in this season (Table 3). Burrowing was more likely to occur in low-medium levels of flow permanence and high salinity (lighter grey region of Fig. 3) and also in streams with high flow permanence and low salinity (lighter grey region: top left corner of Fig. 3).

Some of these interactions seem to be driven by different taxa with the same trait category being abundant in different parts of the space defined by the interaction. With

burrowers in autumn, we found that Oligochaeta (Naididae, Enchytraeidae, Phreodrilidae and Tubificidae) and burrowing groups in the Chironomidae (Chironomini, Podonominae and Aphroteniinae) were involved in the interaction. Burrowing groups in Chironomidae were more abundant in high salinity and longer DSLE (wetter period), and medium levels of salinity and intermediate DSLE (Fig. 4A). In contrast, Oligochaeta were more abundant in medium levels of salinity and longer DSLE (Fig. 4A). Similarly, for the interaction with tegument respiration, dipteran groups (Ceratopogonidae and Simuliidae) were more abundant in high salinity and longer DSLE, while Oligochaeta on the other hand were more abundant in medium levels of salinity and longer DSLE (Fig. 4B). Interaction for collector-gathering was driven by Dipteran groups (Orthocladiinae, Podonominae and Stratiomyidae) and Oligochaeta (Fig. 4C). The dipteran groups were more abundant in streams with medium-high levels of salinity and intermediate to longer DSLE, while Oligochaeta were abundant in medium levels of salinity and longer DSLE (Fig. 4C).

With burrowers in spring, we found that Oligochaeta and burrowing Chironomidae were involved in the interaction between flow permanence (flowPerm) and salinity. Burrowing Chironomidae were more abundant in low-high levels of salinity, and intermediate to higher levels of flowPerm. In contrast, Oligochaeta were only more abundant in low-medium levels of salinity and longer DSLE (Fig. 4D).

Table 3. Significant Spearman rank correlations between macroinvertebrate traits and predictor variables in autumn and spring. Probability values (*P*-values) are in parentheses with correlation values written against the *P*-values. Abbreviations for predictor variables are listed in Table 1 (Cond: salinity; DSLE: number of days since last low flow event; Q75_30: high flow event > 75th percentile in the previous 30 days; and, flowPerm: flow permanence). Instead of correlation values for the interactions, we rather visualise their relationships with trait categories using heat maps derived from the GAMMs.

Abbreviation	**Trait category**	**Cond**	**DSLE**	**flowPerm**	**Cond × DSLE**	**Cond × flowPerm**
			Autumn			
Flgt2	Strong adult flying strength	0.24 (0.012)	-0.21 (0.046)			
Resp3	Plastron, spiracle (aerial respiration)	0.45 (0.015)	-0.38 (0.020)			
Resp2	Gill respiration	-0.19 (0.032)	0.30 (0.021)			
Rheo3	High rheophily		0.21 (0.031)	0.25 (0.028)		
Ther1	Cool eurythermal		0.28 (0.013)	0.40 (0.028)		
Habi1	**Burrower**				(0.034)	
Trop1	**Collector-gatherer**				(0.045)	
Rep3	Ovoviviparity	0.45 (0.043)				
Resp1	**Tegument respiration**				(0.014)	
Trait state	**Meaning**	**Cond**	**Q75_30**	**flowPerm**	**Cond × Q75_30**	**Cond × flowPerm**
			Spring			
Volt1	Univoltinism		0.19 (0.021)			
Volt3	Multivoltinism	021 (0.035)				
Crwl3	High crawling rate		-0.21 (0.003)			
Habi1	**Burrower**					(0.021)
Sal3	Medium-high sensitivity	-0.11 (0.037)				
Mat3	Slow maturation			0.29 (0.022)		
Meta3	Holometaboly		0.16 (0.050)			
Shpe1	Streamlined (flat, fusiform)		0.18 (0.048)			

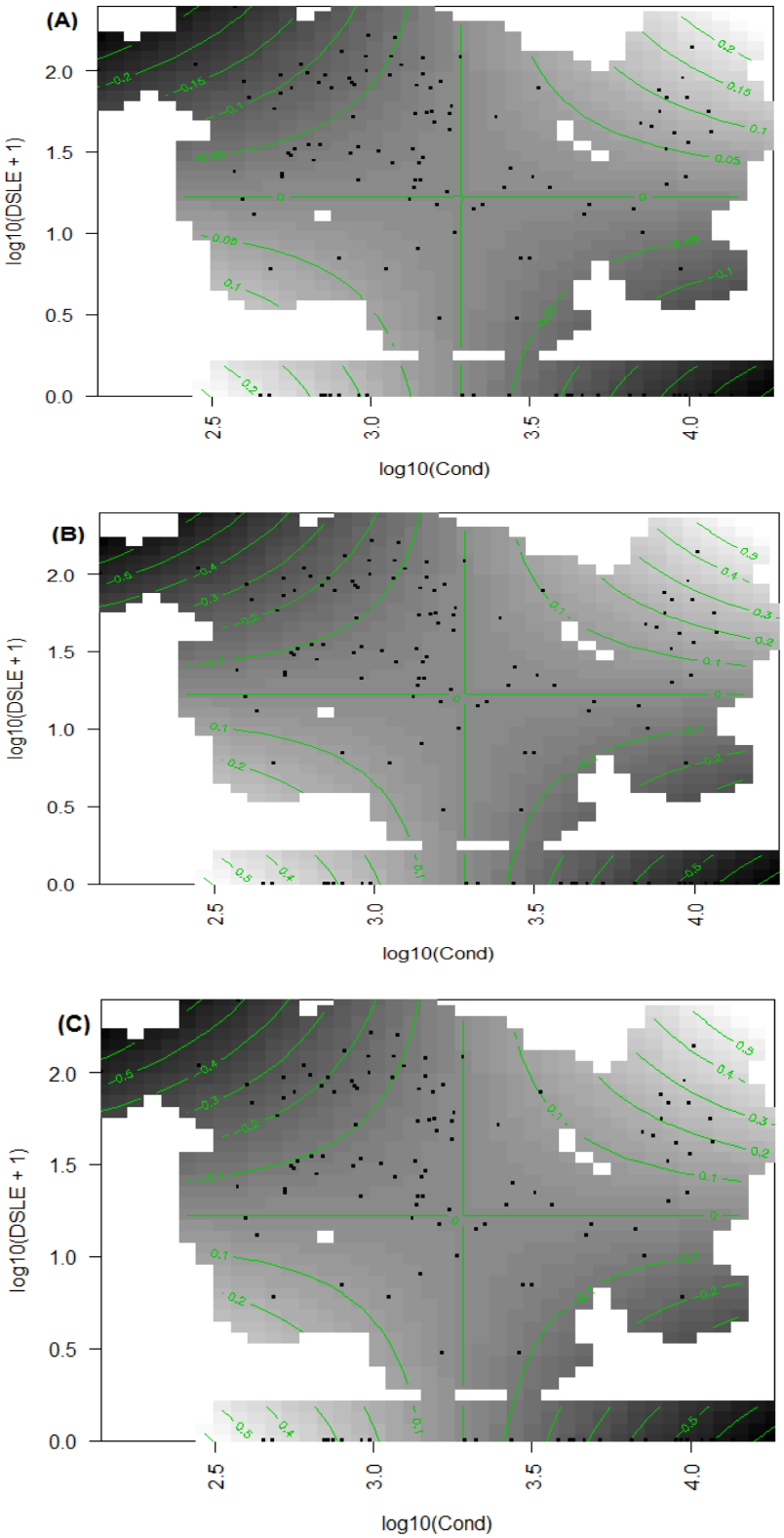


Fig 2. Heat maps of the interaction between salinity (cond) and number of days since last low flow (DSLE) (both variables are on a logarithmic scale) from the GAMM model for (A) Habi1 (burrower), (B) Resp1 (tegument respiration), and (C) Trop1 (collector-gatherer) in autumn. The contours represent the centred abundance of the trait category. The darker regions of the contours denote negative values while the lighter grey areas denote positive values for the trait category. Numerals on contours are centred values for the trait category while the dots represent data points. The white areas indicate the absence of data.

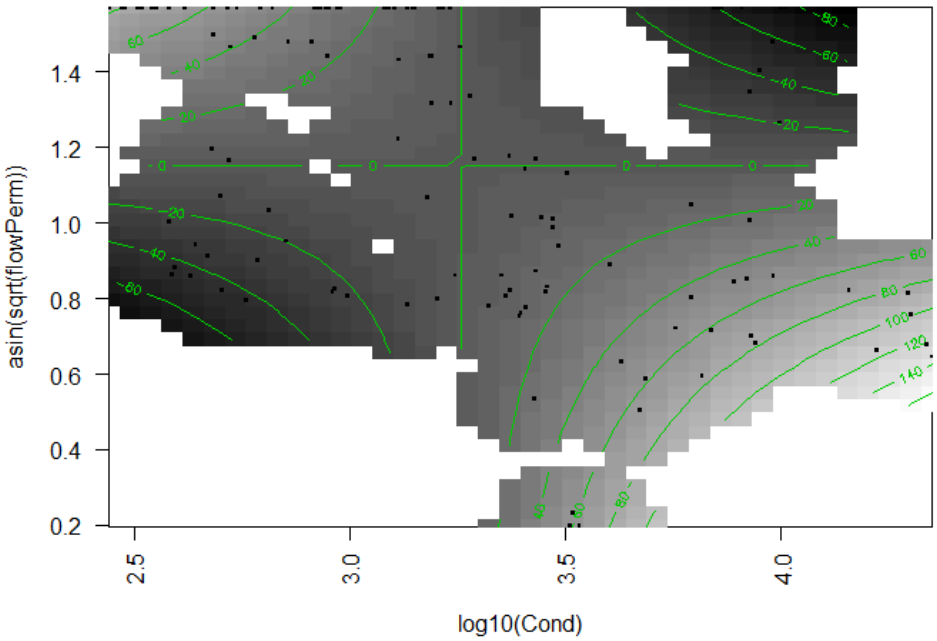
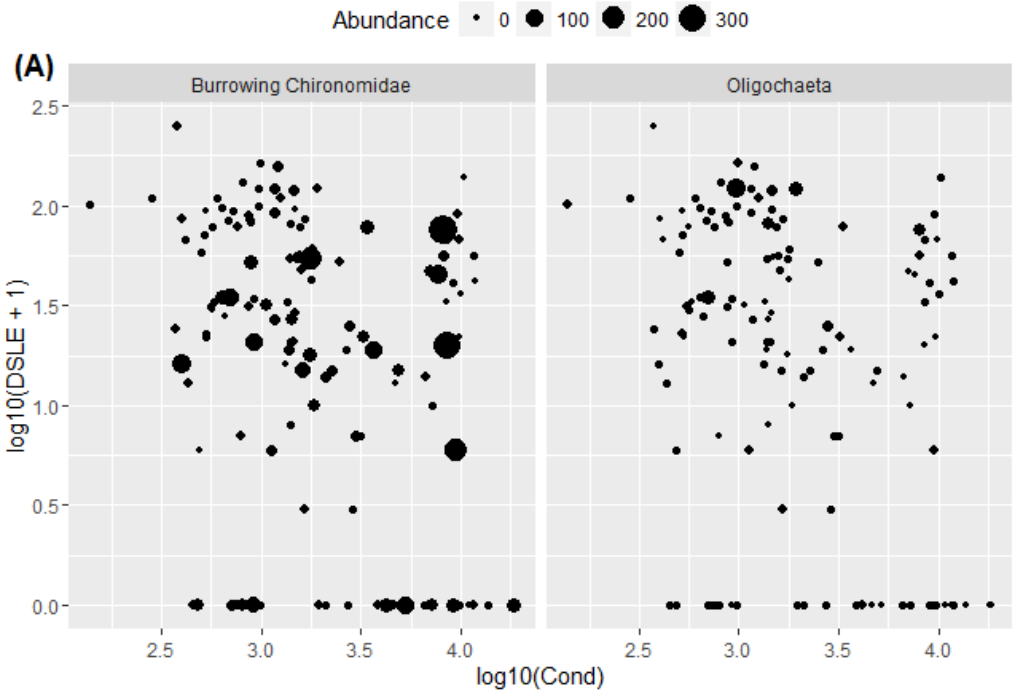
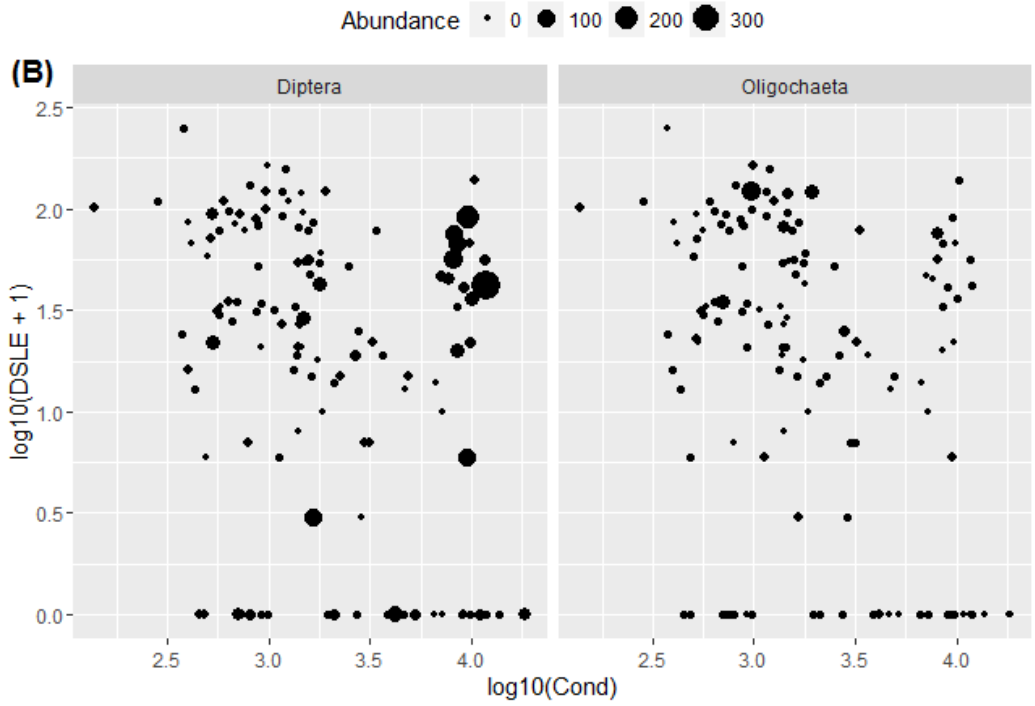


Fig 3. Heat map of the interaction between salinity (cond) and flow permanence (flowPerm) (variable is square-root and arcsine transformed) from the GAMM model for Habi1 (burrower) in spring. The contours represent centred abundance of the trait category. The darker regions of the contours denote negative values while the lighter grey areas denote positive values for Resp1. Numerals on contours are centred values for Resp1 while the dots represent data points. The white areas indicate the absence of data.


Abundance 0 100 200 300
(A)
Burrowing Chironomidae
Oligochaeta
log10(DSLE + 1)
log10(Cond)


Abundance 0 100 200 300
(B)
Diptera
Oligochaeta
log10(DSLE + 1)
log10(Cond)

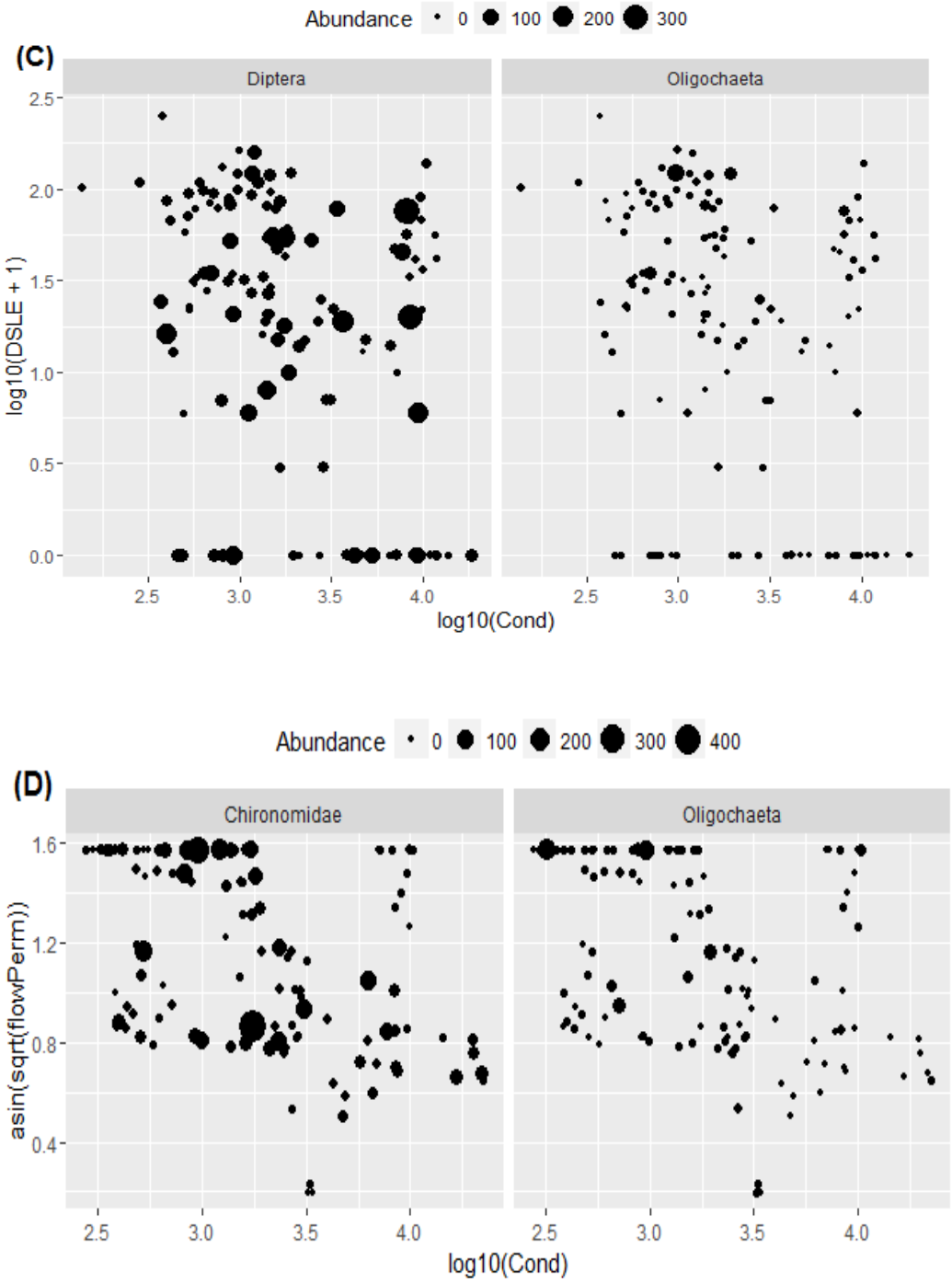


Fig 4. Autumn Bubble plots showing the abundance and distribution of macroinvertebrate groups for the interaction between salinity (Cond) and number of days since last low flow (DSLE) from the GAMM model for (A) Habi1 [burrowers (Oligochaeta: Naididae, Enchytraeidae, Phreodrilidae and Tubificidae; Chironomidae: Chironomini, Podonominae and Aphroteniinae)], (B) Resp1 [tegument respiration (Diptera: Ceratopogonidae and Simuliidae); Oligochaeta], and (C) Trop1 [collector-gatherers (Diptera: Orthocladiinae, Podonominae, Stratiomyidae) and Oligochaeta]. Fig. 4D shows the spring bubble plot for the interaction between Cond and flow permanence (flowPerm) for Habi1 [burrowers (Oligochaeta and burrowing Chironomidae)]. The size of the bubble represents the abundance of the taxa under consideration.

4.5 Discussion

Here, we investigated the effects of flow, salinity and their interaction on macroinvertebrate traits. As expected from previous studies (Chessman, 2015, Szöcs et al., 2014, Walters, 2011), aerial respiration and strong fliers increased with low flows, while gill respiration, high rheophily and cool eurythermality decreased. High flows promoted univoltinism, holometaboly and streamlined body shape while high crawling rate decreased. Multivoltinism, ovoviviparity, aerial respiration and strong fliers responded positively to increases in salinity, whereas gill respiration and medium-high salinity sensitive taxa responded negatively. Interestingly, two of three inconsistently behaving traits in the literature (burrowing and tegument respiration) were associated with interaction between a flow variable (days since low flow event) and salinity. The third trait, collector-gathering, was also associated with an interaction between flow and salinity. However, in other studies, collector-gatherers have increased in response to low flow (Fenoglio et al., 2007). We suggest that, in temporary streams, an interaction between salinity and flow intermittency may be apparent and, may select for macroinvertebrates with traits that enable them to be resilient (ability to recover following high or low levels of salinity and flow; e.g. strong flying strength trait enables macroinvertebrates to escape harsh environmental conditions only to return when conditions become favourable) or resistant (ability to survive in high or low levels of salinity and flow; e.g. ability to burrow) (Poff, 1997).

Although biological traits have been cited to have the potential to identify the relative importance of different stressors or disentangle the effects of multiple stressor on stream systems (Statzner and Bêche, 2010, Wooster et al., 2012), however, our study suggests that some traits (e.g. burrowing, tegument respiration and collector-gathering) may not be accurate indicators of salinization alone because these same traits also responded to effects from flow intermittency. These traits are rather indicative of multiple stressor effects. Thus, to foster the operative use of traits to disentangle the effects of multiple stressors on ecosystems, there is the need for the development of a better mechanistic understanding of how specific stressors (e.g. flow and salinity) act as trait filters, potentially through the use of experiments (Charvet et al., 2000, Villeneuve et al., 2015), to ensure that each of the stressors is strong enough to produce clear trait responses.

The use of invertebrate traits have been proposed as an approach to use to assess effects of flow (Walters, 2011) or salinity (Kefford et al., 2012) because taxonomic-based approach is limited by the variability of taxa across different biogeographic regions (McGill et al., 2006). However, we found site to be still the strongest predictor of trait composition, although this effect was not as strong as the effect of site on taxonomic composition (Supporting Fig S2). Interestingly, flow and salinity explained a relatively greater proportion of the variance in trait space (27.3% in autumn, 36.7% in spring) than the proportion of variance explained in taxonomic space (20.9% in autumn, 27.7% in spring) for this study (Botwe et al., 2015). Thus, examining traits in our study performed moderately better as indicators of environmental conditions than focussing on taxa.

4.5.1 Traits response to separate effects of salinity and flow

As reported elsewhere (Díaz et al., 2008, Piscart et al., 2006, Szöcs et al., 2014), increasing salinity promoted ovoviviparity, multivoltinism, fliers and aerial respiration, while gill respiration and taxa with medium-high physiological sensitivity to salinity decreased. However, Piscart et al. (2006) did not observe a change in gill respiration. This could be due to two reasons. First, in our study, we focused only on the most discriminating traits (as identified by the BVSTEP) whereas Piscart et al. (2006) tested for statistically significant differences for all trait categories they used, and corrected for multiple comparisons, which might have caused them to lose power to detect this trait. Secondly, Piscart et al. (2006) covered a much smaller range of salinities (predominantly Na^+ and Cl^-) (0.21g L^{-1} to 2.60g L^{-1}, equivalent to 435 µS cm^{-1} to 4,860 µS cm^{-1}) than our study (0.07g L^{-1} to 14.34g L^{-1} equating to 139 µS cm^{-1} to 23,700 µS cm^{-1}).

The ecological explanations supporting these trait relationships with increasing salinity are as follows. Ovoviviparity is favoured because it protects the young by isolating the eggs from external elevated salt concentrations (Piscart et al., 2006). Multivoltine life cycles are held to be advantageous since it increases the chances of survival of offspring (Kefford et al., 2004). Young individuals commonly have a lower salinity tolerance than older stages (Kefford et al., 2004), and are likely to grow more slowly in response to flow pulses of high saline water, thus making adult females produce more than two generations per year to increase chances of survival of their offspring. Unsurprisingly, the number of both medium and highly salt-sensitive taxa decreased with increased salinity, as would be expected given the physiological basis of these two trait categories (Schäfer et al., 2011).

Gill-respiring taxa also decreased in salty streams, most likely because salty streams commonly have low dissolved oxygen concentrations which should favour macroinvertebrates with morphological structures (e.g. spiracles, siphons, plastrons) to breathe atmospheric oxygen (Chapman et al., 2004, Hinton, 1976).

As found elsewhere (Chessman, 2015, Horrigan and Baird, 2008, Walters, 2011), high rheophily, cool eurythermality and gill respiration decreased during low flows (which are typified by high water temperatures and low oxygen concentrations), while aerial respirers and strong fliers increased. Unsurprisingly, as flow permanence increased, high rheophily and cool eurythermality also increased. The ecological explanations supporting these trait relationships with reduced flows are as follows. The warmer conditions that characterise low flows might have reduced the abundance of thermally sensitive taxa, while the cooler conditions when flow permanence increased could have allowed for such taxa to rebound. Furthermore, reduced flow often leads to decreases in overall habitat availability and an increased proportion of pool habitats. These changes would favour taxa with the ability to move as habitat contracts, e.g., taxa with strong flying strength, high crawling rate or burrowers, and taxa adapted to the warmer temperatures and lower oxygen levels in pools (Walters, 2011).

Additionally, traits characteristic of slow maturation (holometaboly and univoltinism) were favoured during increased flow permanence, which accords with the common pattern in temporary streams (Chessman, 2015, Williams, 1996). This is because invertebrates with slower maturation may not recruit more successfully during low flows as they may not be able to complete development from egg to adult during brief flow episodes or in residual pools. During favourable environmental conditions (e.g. high flows), invertebrates may tend to invest in fewer but larger eggs to improve fitness of their offspring, and this trade-off has been reported by Berrigan (1991). It was therefore not surprising that we found an increase in univoltinism (only one brood or generation per year) during high flows in our study.

High crawling rate and strong fliers are other traits that are supposedly an adaptation to low flows, and these increased with lower flows in our study as they did for James et al. (2009). High crawling rate may enable organisms to follow retreating water to avoid stranding (Stanley et al., 1994), and strong flying strength enables macroinvertebrates to escape harsh environmental conditions only to return when conditions become favourable (Brooks et al., 2011).

4.5.2 Interactions between flow and salinity

Interestingly, we found burrowing, tegument respiration and collector-gathering were associated with interactions between salinity and flow, and there are contradictory results reported for these traits in the literature. Walters (2011) found that a low flow disturbance in Connecticut, North America promoted burrowers, while Bonada et al. (2007) reported the opposite response during an observational study of traits response to low flows in streams in Catalonia, Spain. Similarly, Brooks et al. (2011) found that an experimental low flow disturbance in New South Wales, Australia, promoted tegument respiration , while Bonada et al. (2007) reported the opposite response. The stream sites used in these studies were located in catchments that were also affected by natural salinization. However, these studies did not examine interactions between salinity and flow. We therefore suspect that, there might have been interactions between salinity and flow, separately in these studies which might have resulted in these two traits responding differently to low flows. Collector gathering has previously shown an increase in response to low flows (Fenoglio et al., 2007). However, Brooks et al. (2011) in assessing invertebrates' response to low flows resulting from water abstraction, did not find a change in response of collector-gatherers. Perhaps, this may be due to the fact that, interactions between flow and salinity within their study system must have masked the effect of a single stressor (e.g. flow), thereby not being able to detect response of collector-gatherers to low flow conditions.

The interactive effects of flow and salinity on macroinvertebrate traits we found in our study suggest that some traits (e.g. burrowing, tegument respiration and collector-gathering) may not be accurate indicators of conditions in stream ecosystems as these traits responded to effects of both flow and salinity. For example, ovoviviparity may be an accurate indicator of effects of salinity, as this trait responded to only salinity (and not flow) effects in my study, just as was also reported by Díaz et al. (2008), Piscart et al. (2006), Szöcs et al. (2014).

Some of these interactions seem to be driven by different taxa with the same trait category being abundant in different parts of the space defined by the interaction. For example, we found that burrowing groups in Chironomidae were more abundant in high salinity and longer DSLE (wetter period), and medium levels of salinity and intermediate DSLE. In contrast, Oligochaeta were more abundant in medium levels of salinity and longer DSLE. Similarly, for the interaction with tegument respiration, dipteran groups

(Ceratopogonidae and Simuliidae) were more abundant in high salinity and longer DSLE, while Oligochaeta on the other hand were more abundant in medium levels of salinity and longer DSLE. The differential response of these taxa to interactions between salinity and low flows in their respective trait groups (as indicated by the bubble plots) appear to have caused the interactions we observed. The ecological explanations supporting the association of these inconsistently behaving traits with interactions between salinity and flow are as follows. Previously, the ability to burrow has been linked to desiccation resistance (resulting from low flows) for some aquatic insects in intermittent streams (Acosta and Perry, 2001, Boulton and Stanley, 1995). However, we found that burrowing groups in the Chironomidae were abundant in streams with high salinity and longer DSLE (wetter periods), suggesting that burrowing may not only be an adaptation to survive desiccation but also an adaptation to withstand being washed away by relatively faster water currents during high flows. Furthermore, Oligochaetes and some chironomids have been found in salty streams (including hyper-saline running waters) regardless of the hydrological regime (i.e. either low or high flows) (Gillett et al., 2007).

The increase in the abundance of tegument respiring taxa (e.g. Ceratopogonidae) during high salinity and longer DSLE may be due to an increase in the deposition of fine sediments (Larsen et al., 2011) carried along by the relatively higher flow rate, while Simuliidae may have also increased because of the relatively increased flows (Srisuka et al., 2015) to meet their requirement of filter feeding. Collector-gatherers (e.g. Orthocladiinae) may have increased in abundance in streams with low-high levels of salinity and intermediate to longer DSLE, because of the availability of abundant food source [e.g. fine particulate organic matter (FPOM)] in the pool habitats, which might have been caused by an increased activity of shredders (Cummins et al., 2005, Merritt et al., 2002). Unsurprisingly, burrowers in spring (e.g. Podonominae) may have increased in abundance in streams with low-medium levels of salinities and increased flow permanence due to the cooler conditions associated with increased sustained flows. Podonominae tend to cool stenothermy and are usually lotic (Wirth and Sublette, 1970).

4.6 Conclusions

Our study has demonstrated congruence across studies regarding how particular traits response to flow intermittency and gradients of salinity, thus generally conclusions can be drawn regarding these responses. We suggest that hydrological alteration to ground water

may be associated with salinity increases and its influence on trait composition. These changes in salinity and hydrology, which could be the result of natural variability inherent in temporary streams, may filter for macroinvertebrates with traits that enable them to be resistant or resilient to high or low levels of salinity and flow (Brock et al., 2005).

This study has also shown that interactive effects may underlie inconsistencies reported in literature regarding the response of some traits (burrowing, tegument respiration and collector-gathering) to flow or salinity, and that these interactions may be apparent in temporary streams. The interactions seem to be driven by the differential response of different taxa with the same trait category being abundant in different parts of the space defined by the interaction. Our findings suggest that, although traits have the potential to be used as indicators of multiple stressor effects on stream ecosystems, some traits may not be accurate indicators as they response similarly to multiple stressors. Thus, developing a better mechanistic understanding of how specific stressors (e.g. flow and salinity) act as trait filters, potentially through the use of experiments, may be warranted, to ensure that each of the stressors is strong enough to produce clear trait responses.

Even though, there was a significant effect of site on the trait composition, traits were less sensitive to geographic variation than that of taxa across our study sites. Furthermore, flow and salinity explained modest amounts of variation in the trait matrix relative to the variance explained in the taxonomic space. This suggests that examining traits in our study performed moderately better as indicators of environmental conditions than focussing on taxa.

4.7 Acknowledgements

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4.8 Author contributions

PKB, SC, LAB and RM conceived and designed the project. PG, PM and CM performed the experiments. PKB wrote the preliminary manuscript. PKB, SC, LAB and RM analysed the data. PG, PM and CM contributed reagents/ materials/ analysis tools. All authors discussed analysis and results, edited manuscript text, and gave final approval for publication.

4.9 Supporting information

Fig S1. Map of South Australia, showing the distribution of major land-uses and the sampling sites. Circles represent study sites; catchment boundaries upstream of study sites denoted by black lines; coastline and state borders in grey lines; thin black lines represent boundaries to NRM regions.

Fig S2. Canonical Analysis of Principal Component (CAP) ordinations overlaid with clusters to show the relative sensitivities of (A) taxonomic, and C) trait composition to geographic variation across multiple catchments. Ovals around site symbols show how sites cluster, and represent percentage similarity among the different clusters of sites. Different symbols represent different sites used in the study and their meaning are listed in Table S1 (▲: Bremer River; ▼: Finniss River; ■: First Creek; ♦: Hill River; ●: Hindmarsh River; +: Kanyaka Creek; ×: Light River; *: Marne River; Δ: Myponga River; ∇: North Para River; □: Rocky River; ◊: Scott Creek; ○: Torrens River)

Table S1a. List of sites surveyed with their respective ionic proportions. Abbreviations and symbols represent: R (River); C (Creek); Agric (percent agriculture); Sym (symbol); the major cation and anion are sodium and chloride ions respectively.

Table S1b. List of sites surveyed with their respective mean ionic concentrations. Abbreviations and symbols represent: R (River); C (Creek); S.E (standard error)

Table S2. Description of 26 traits in 75 modalities applied to 811 genera/ species in 185 families of South Australia freshwater macroinvertebrates categorized into four trait groups (bolded). The letter in each "Code" refers to the trait and the suffixed number refers to the trait state.

Table S3. Spearman's correlations coefficients (ρ) between flow variables and salinity for the 13 sites. Abbreviations for predictor variables are listed in appendix 2. Bold face indicates ρ > |0.70| between variables for which reason flowPerm was chosen over med.CTF

Table S4. Results of the multivariate generalized linear mixed models (MGLMM) for the 13 sites based on the trait states identified by BVSTEP for autumn and spring. Bold numbers indicate significant *P*-values. *df* indicate degrees of freedom.

Competing financial interests: The authors declare no competing financial interests.

Chapter 5

Contrasting taxonomic and traits response of stream macroinvertebrates in temporary streams

River the Hoëgne in Belgium (Photo by Jan-Arie van den Boogert)

Chapter 5 is in preparation

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5.1 Abstract

Do functional traits convey more or different information about community patterns and community-environment relationships than focussing on the taxa? Both of these approaches (i.e. taxonomic and trait-based) have been used separately in bioassessment, but studies that simultaneously contrast the two approaches in response to abiotic factors are rare. We used multivariate approaches to investigate within-site temporal variability in macroinvertebrate compositional and trait data spanning 13 years of sampling in perennial and intermittent streams during autumn and spring in South Australia. We examined how flow and salinity environmental variables predicted variability in taxonomic and trait composition, and contrasted models to determine whether the models would perform substantially better in predicting functional traits than taxa. The top models predicting invertebrate structure explained more variation in trait space than taxa; in some instances a lot more (19% and 40%), and others, smaller gains (2 and 3%), although there was no discernible pattern related to whether or not the stream was perennial. For the most important traits in these top-ranked models, the differences in variance explained with taxa was associated with greater numbers of macroinvertebrate families with lower taxonomic dominance per trait. Thus, more taxa sharing the same trait may reduce the redundancy associated with using taxonomic identities, so that the trait-space has greater explanatory power than the taxonomic space. Further, trait composition was more similar between seasons than taxonomic composition because there was more turnover in taxa than trait structure. This study suggests that functional traits performed better as indicators of environmental conditions than focussing on taxa, although the gains in variance explained varied greatly. The greater similarity of trait composition between seasons than taxonomic composition also suggests that some traits are equally advantageous in both the dry and wet seasons. Thus, a management outcome of this research is that biomonitoring could be reduced to one sampling event per year if focussing on traits for environmental management guidelines is sufficient to address the monitoring goal.

5.2 Introduction

Variability in ecological communities arises from both spatial and temporal gradients in biotic and abiotic factors that control their abundance and distribution (Poff, 1997, Townsend et al., 1997). Many studies have examined the taxonomic spatial and temporal variability of macroinvertebrate communities (e.g. Amoros and Bornette, 2002, Botwe et al., 2015), but the low variance explained when relating aquatic macroinvertebrate composition to

environmental factors, and the biogeographic effects that characterizes taxonomic-based analysis have led to proposals to use functional traits to augment taxonomic-based analyses (McGill et al., 2006, Poff et al., 2006).

There are three main claims made to support the use of traits. Firstly, traits may overcome biogeographic effects (McGill et al., 2006) because taxonomic studies are restricted in their extendibility across studies owing to biogeographic variation in species identities across different regions (Townsend et al., 1997). Secondly, examination of traits may help improve our understanding of the casual mechanisms by which environmental disturbances affect ecological communities (Townsend et al., 1997). This is because the use of taxonomic identities often do not improve our understanding of the causal mechanisms by which impacts occur, except in situations where there is detailed background information on the taxon's physiology (Dolédec et al., 2000, McGill et al., 2006). Thirdly, environmental factors may explain more of the variation in trait composition than taxa (McGill et al., 2006), because taxonomic identities varies more geographically and temporally than traits (Tomanova and Usseglio-Polatera, 2007, Townsend et al., 1997). This variability potentially creates redundancies which, when relating macroinvertebrate taxonomic composition to environmental factors, may lead to low variance explained (Harris and Heathwaite, 2012, Hirst and Kilpatrick, 2007).

However, the research presented in our preceding chapter (i.e. Chapter 4 of this thesis) suggested that traits were still sensitive to biogeographic variation. To continue to evaluate traits in comparison to taxa, we instead focussed on within-site analyses. By focussing within sites, we are able to overcome biogeographic signals that affect taxonomic and trait composition. Thus, within-site analysis may enable us assess whether models will predict traits substantially better than taxa, explicitly testing the third claim for the use of traits.

Our study area in southern South Australia constitutes a gradient from Mediterranean through to arid, warm temperate climates, with flow conditions ranging from permanent to ephemeral. Temporary streams are abundant across this region (Laut et al., 1977) and includes seasonal patterns in flow, salinity and temperature that result in hot, dry summers and cool, wet winters. Aquatic invertebrates in this system have been exceptionally well sampled (13 sites, sampled bi-annually for 13 years) and thus present an ideal system to examine within-site temporal variability, and contrast taxonomic with trait-based approaches.

We focussed on two types of stream conditions: perennial and intermittent streams in order to consider the generalizability of our study focus across stream types. Here, we test three key predictions. We predicted that: (1) trait composition will be more similar between seasons than taxa, because there is more turn over with taxa over time than with traits (Bêche et al., 2006, Ruhi et al., 2009); (2) In both taxonomic and trait composition, intermittent streams will be less similar between seasons than perennial streams because intermittent streams are more likely to lose species by chance during dry periods than perennial streams (Bonada et al., 2007, Brooks and Haeusler, 2016, Lake, 2003); (3) by focussing within-sites, models will perform substantially better for predicting invertebrate trait composition than taxa in response to abiotic factors (e.g. flow and salinity).

5.3 Methods

5.3.1 Study sites

The study was conducted in South Australia. We studied four sites: two perennial (Finniss River and Scott Creek) and two intermittent (Hill River and Kanyaka Creek) streams, because these streams had the most complete flow records, and spanned the full range of flow conditions in our study system.

Finniss River (FR) has a catchment area of 187.2 km^2. It is a large stream in the southern Mount Lofty Ranges that rises east of Yundi and flows in an easterly direction where it eventually discharges into the Lower Murray, northeast of Goolwa (Armstrong et al., 2003). Scott Creek (SC) has a catchment area of 26.7 km^2 and is a sub-catchment of the Onkaparinga River to the south east of Adelaide. Grazing comprises the major land use in this catchment with native vegetation existing on the steeper slopes of the catchment (Deane, 2012). The climate of both catchments are typically temperate and rainfall tends to occur in winter and spring with the bulk of the rainfall in the winter months (Harrington, 2004) [Supporting Fig. S1].

Kanyaka Creek has a catchment area of 177.2 km^2 and rises near Hawker and discharges into Willochra Creek, about 5 km north from Simmonston. The major land uses in this catchment, upstream from the site sampled, are grazing modified pastures (84%) and grazing natural vegetation (15%) (Deane, 2012). Hill River has a catchment area of 245.8 km^2 and occurs approximately 3 km east of Penwortham and flows north to join Yakilo Creek

to form the Broughton River (Deane, 2012) [Supporting Fig. S1]. Both streams are highly intermittent (usually 100-200 zero-flow days per year) (Kennard et al., 2010).

5.3.2 Macroinvertebrates sampling

The macroinvertebrate samples used in our analyses form part of the Australian Rivers Assessment System (AusRivAS) (Davies, 2000) of which South Australia has been part since 1994. The database includes standardised records of benthic macroinvertebrates and associated environmental variables. Annual sampling was conducted in two seasons (autumn and spring) to avoid periods when some streams may dry out in summer and low macroinvertebrate activity during winter. We used data collected for 13 years from 1994 to 2007 (excluding 1996 owing to a hiatus in funding).

Macroinvertebrates were collected using standardised AusRivAS protocols which consisted of sampling approximately 5 m^2 area of edge and pool habitats within each 100 m study site using a 250 µm mesh triangular dip net. Sampling involved vigorously kicking the substrate and sweeping the net over a total bank length of 10 m using sequential short sweeping movements at right angles to the bank and, sweeping under overhanging or emergent vegetation (Davies, 2000). Collected macroinvertebrates were preserved in ethanol on site, transported to the laboratory, and subsampled (where a minimum of 10% of the sample was counted and identified using dissecting and compound microscopes), and the residue scanned for rare taxa (Davies, 2000, Simpson and Norris, 2000). This approach ensured observer bias was minimised when counting individuals compared to alternative live-pick approaches included in the AusRivAS protocols, and it also provided an accurate estimation of the abundance of cryptic taxa. Taxa were identified to the lowest taxonomic level, given available keys, life-history stage and condition. This was most often to genus or species level. Voucher specimens of all taxa were retained as a reference collection at the South Australia Museum and Australian Water Quality Centre (AWQC).

5.3.3 Traits

We used 75 biological traits grouped into four categories (life history, mobility, ecology and morphology) to describe the functional composition of invertebrate communities (Supporting Table S1). Trait values were assigned at family level (except for the Chironomidae, where traits were assigned at subfamily level) using existing trait databases of Poff et al. (2006), Schäfer et al. (2011) and, where South Australian taxa were not covered in these sources, we

utilised expert opinion from taxonomists and information from *Identification and Ecology of Australian Freshwater Invertebrates* (http://www.mdfrc.org.au/bugguide/, accessed January 2016). The trait databases from Poff et al. (2006) and Schäfer et al. (2011) described the affinity of each family to each category with scores ranging from "0" (no affinity) to "5" (high affinity) using the fuzzy coding approach of Chevenet et al. (1994). We used family level taxonomic resolution (except for Chironomidae, where subfamilies were used) because Gayraud et al. (2003) argued that the functional structure of freshwater macroinvertebrate communities is conserved if taxonomic levels higher than species are used, especially when analysed with multivariate methods.

Phylogenetically linked traits (trait "syndromes") (Bêche and Resh, 2007, Poff et al., 2006) may respond similarly or in tandem to an environmental gradient, creating redundancies that may complicate interpretation (Poff et al., 2006, Poteat et al., 2015). To minimise the impact of these issues, we followed Poff et al. (2006) and Culp et al. (2011) by choosing traits that were highly labile evolutionarily (e.g. rheophily, thermophily) and which were also hypothesised as having mechanistic links with the environmental variables we were considering, flow and salinity (Chessman, 2015, Díaz et al., 2008).

Trait category scores were multiplied or weighted by the abundance of the taxa to obtain a sample-by-trait matrix that contains for each sample the averages of numerical traits over all macroinvertebrate taxa present (Walters, 2011).

5.3.4 Environmental and hydrological data

Salinity was measured *in situ* on each sample date as electrical conductivity at 25 °C in μS cm^{-1} using calibrated water quality meters. Flows in these streams are largely driven by ground water which has accumulated marine-derived salts over long time-scales (Herczeg et al., 2001). In places, clearing of native vegetation and irrigation for agriculture further exacerbates salinity. There are no secondary sources of salinization such as from mining, coastal inundation, industrial discharge or road de-icing, and electrical conductivity is dominated by Na^+ (median: 72 % of cations) and Cl^- (median: 98% of anions), with similar ionic compositions across this region (Supporting Table S2).

All sites had flow-gauges from which mean daily flow data were compiled to calculate flow metrics. Six metrics that described ecologically relevant aspects of the magnitude and frequency of flow (Allan et al., 1997) were calculated in R version 3.2.4 (R

Core Team, 2016) using base functions and those in the "hydrostats" package (Bond, 2015) (Supporting Table S3). These were: median flow (medQ) [magnitude], median cease-to-flow (med.CTF) [spell duration], annual average flow permanence (flowPerm) [magnitude] (Arscott et al., 2010), number of days since last low flow event 365 days prior to sampling (DSLE) [spell duration], number of days since the last high flow event (DSHE) 365 days prior to sampling [spell duration] and high flow disturbance (Q75_30) [magnitude] in the previous 30 days, which is classified as number of flow events ≥ 3 times the 75th percentile daily discharge. We used three times the 75th percentile discharge [modified from Greenwood and Booker (2015), because the median discharge of some of our streams were zero] as an indicator of high flow disturbance because flows exceeding this measure have been shown to reset invertebrate community structure (Arscott et al., 2010, Booker, 2013, Greenwood and Booker, 2015). A low flow event was defined as the 80th percentile exceedence non-zero daily flow rate (Smakhtin, 2001, VanLaarhoven and Van der Wielen, 2009). These flow variables were chosen because they captured independent aspects of flow duration and magnitude.

We examined a wider range of flow variables initially, but after we examined co-linearity among normalized salinity and flow variables using Spearman's correlation coefficient (ρ) and scatter plot matrices, we selected variables from correlated sets that were likely most proximally related to the traits (Clarke and Warwick, 2001), in order to reduce redundancy. For example, we found that annual average flow permanence (flowPerm) was highly negatively correlated (ρ = - 0.76) with median cease-to-flow (med.CTF), and flowPerm was used instead of med.CTF because we envisaged invertebrate traits responding to flow permanence rather than the median period of cessation of flow (Arscott et al., 2010) (Supporting Table S4). The final selected flow variables were not strongly correlated with one another (all ρ < |0.54|), nor was salinity strongly correlated with any of these flow variables (all ρ < |0.38|, Supporting Table S4).

5.3.5 Statistical analyses

5.3.5.1 Within-site temporal changes in macroinvertebrate communities and trait composition

To explore temporal changes within sites in assemblage and trait composition with two factors (season and year), permutational multivariate analysis of variance (PERMANOVA)

(Clarke and Warwick, 2001) based on Bray-Curtis similarity [for taxonomic, (Bray and Curtis, 1957)] and Gower similarity [for traits, (Gower, 1971)] of log-transformed data resolved at family level was used. Formal testing was undertaken using the PERMANOVA+ (v1.0.6) software extension to PRIMER (Clarke and Gorley, 2006). A two-dimensional (2D) Non-metric multidimensional scaling (NMDS) (Kruskal, 1964) was used to visualize temporal changes in assemblages and trait composition within sites, between seasons and across years. Where the NMDS stress was > 0.15, diagnostic analysis using a three-dimensional (3D) NMDS and Principal Coordinate Analysis (PCO) were employed to check whether the arrangement of samples were real or just arbitrary placement in space (Clarke, 1993, Gower, 2014). Data from perennial and intermittent sites were analysed separately.

To examine which approach (taxonomic or trait-based) or stream type (i.e. perennial and intermittent sites) were more similar between seasons within sites, pairwise tests for seasons using PERMANOVA (9999 permutations) was conducted for each site within each of the taxonomic and trait data sets (Clarke and Warwick, 1994, Clarke and Warwick, 2001). These were complemented by inspecting trait categories that contributed the greatest proportion to the average dissimilarity between seasons for intermittent and perennial sites, by using the Similarity Percentages (SIMPER) routine in PRIMER (Clarke and Warwick, 2001, Warfe et al., 2013).

5.3.5.2 Relationship of assemblage and trait composition to flow and salinity

We used flow metrics and salinity to examine which variables may be correlated with macroinvertebrate community assemblages and trait composition. A distance-based linear model (DistLM) (Clarke and Gorley, 2006, Warfe et al., 2013) with single and all pairwise combination of predictors (flow metrics and salinity), using Akaike Information Criterion [(AICc), corrected for small sample sizes] as the selection criterion, was used to derive the most parsimonious models predicting macroinvertebrate communities and trait composition. The DistLM enabled us to identify predictor variables (on a normalised scale) that contributed significantly to the temporal patterns observed in the assemblage and trait structure, as well as determine how much variation was explained by each predictor. We calculated delta AICc (ΔAICc), Akaike weights (w) and variable importance for each model using Anderson (2008). Diagnostic analysis using variance inflation factors (VIF) were employed to check for multicollinearity (correlations between predictor variables): this was not an issue because all the VIFs were < 2.5 (Berk, 2003).

The models were complemented by inspecting a subset of trait categories that best correlated with patterns in the predictor variables of the top model (as represented by the distance matrix between predictors) (Clarke and Ainsworth, 1993). This was done using the BVSTEP (Best Subset of Environmental Variables with maximum Correlation with Community Dissimilarities) procedure in PRIMER (Clarke and Gorley, 2006, Clarke and Warwick, 2001) and tested using 9999 permutations (Clarke and Ainsworth, 1993, Clarke and Gorley, 2006, Clarke and Warwick, 2001). To assess how the taxa may be mapping on the strongly responding traits, the number of taxa in each of these trait categories was computed. Additionally, we calculated Simpson's dominance (*D*) for the macroinvertebrates associated with each trait category identified by the BVSTEP procedure using:

$$D = 1 / \sum P^2_i$$

where P_i is the proportion of individuals (relative abundance) in the *i*th taxon (Simpson, 1949). We chose this index because it is one of the most robust dominance measures (Beisel et al., 1996, Magurran, 2013, Morris et al., 2014).

5.4 Results

Overall, 176 samples were collected for the perennial and intermittent sites, and comprised a total of 55,095 individuals from 840 taxa. For the perennial sites, Finniss River and Scott Creek, salinity ranged from 484 – 1920 µS cm^{-1} and range of values for the flow variables included: median flow (0.94 – 15.84 ML/ day), average flow permanence (70 – 100 %), days since low flow event (0 – 304 days), days since last high flow event (0 – 241 days), high flow disturbance (0.72 – 81.41) [Supporting Table S3]. For the intermittent sites, Hill River and Kanyaka Creek, salinity ranged from 1470 – 22427 µS cm^{-1} and range of values for the flow variables included: median flow (0 – 1.45 ML/ day), average flow permanence (2 – 72 %), days since low flow event (0 – 179 days), days since last high flow event (0 – 301 days), high flow disturbance (0 – 12.26) [Supporting Table S3].

5.4.1 Within-site temporal changes in macroinvertebrate communities and trait composition

Multivariate analysis showed strong seasonal and annual effects on macroinvertebrate taxonomic structure ($P < 0.05$) [Fig. 1, Table 1]. Stresses on the two-dimensional (2D) NMDS for taxonomic composition were high (> 0.15). However, reanalysis using three-

dimensional (3D) NMDS did not reduce stresses substantially (ranged from 0.11 to 0.14 for intermittent and perennial streams), and also revealed similar temporal patterns to the 2D NMDS. This suggests that the patterns observed in taxonomic space using a 2D NMDS were not arbitrary (Clarke, 1993, Gower, 2014). By contrast, NMDS based on traits had acceptably low stresses in 2D, and showed no seasonal effect, except for intermittent Kanyaka Creek ($P < 0.001$) [Fig. 2, Table 1]. Neither trait nor taxonomic community composition showed clear trends in their trajectories across years, suggesting that sites did not change in any consistent direction over time (Fig. 1 and 2). However, there were strong annual effects on taxonomic composition, but there were no significant inter-annual differences for trait-based analyses (Table 1).

As expected, taxonomic composition was less similar between seasons within sites (similarity: Finniss River, 59.3%; Scott Creek, 56.8%; Hill River, 45.3%; and Kanyaka Creek, 52%) than for traits (similarity: Finniss River, 92.6%; Scott Creek, 88.2%; Hill River, 77.1%; and Kanyaka Creek, 82%). Further, within both taxonomic and trait analyses, intermittent sites (Hill River and Kanyaka Creek) were less similar between seasons than perennial sites (Finniss River and Scott Creek).

Results from the SIMPER analysis revealed small differences in trait turnover within perennial (dissimilarity: 3% more in Finniss River than Scott Creek) and intermittent streams (dissimilarity: 2% more in Kanyaka than Hill River) [Supporting Table S5]. Within sites, a number of trait categories were found to contribute mostly to the average dissimilarity between seasons. For instance, within perennial streams, trait categories that primarily drove the average dissimilarity between seasons in Finniss River were mainly skating, respiration via gills and large body size, while flattened body shape, slower maturation and high rheophily were more important in Scott Creek (Supporting Table S5). Within intermittent streams, skating, faster maturation and very short life cycle differed between seasons in Kanyaka Creek, while skating, strong swimming ability and aerial respiration contributed most to the average dissimilarity between seasons in Hill River (Supporting Table S5). Between the two intermittent streams (i.e. Hill and Kanyaka Creek), skating was the only common trait that differed between seasons.

Table 1: Results of permutational multivariate analysis of variance (PERMANOVA) on taxonomic and trait composition of individual sites for perennial and temporary streams. *F*: pseudo-F statistic; *df*: degrees of freedom. Bold numbers indicate significant *P*-values (< 0.05).

Taxonomic composition				**Taxonomic composition**			
Scott Creek (Perennial)				*Finniss River (Perennial)*			
	df	*F*	*P*		*df*	*F*	*P*
Season	1	2.53	**0.002**	Season	1	2.91	< **0.001**
Year	1	1.91	**0.028**	Year	1	1.65	**0.034**
Residual	18			Residual	18		
Hill River (Intermittent)				*Kanyaka Creek (Intermittent)*			
	df	*F*	*P*		*df*	*F*	*P*
Season	1	2.43	**0.006**	Season	1	4.60	< **0.001**
Year	1	1.96	**0.027**	Year	1	2.59	**0.004**
Residual	18			Residual	18		
Trait composition				**Trait composition**			
Scott Creek (Perennial)				*Finniss River (Perennial)*			
	df	*F*	*P*		*df*	*F*	*P*
Season	1	2.47	0.082	Season	1	0.60	0.510
Year	1	2.71	0.078	Year	1	1.47	0.251
Residual	18			Residual	18		
Hill River (Intermittent)				*Kanyaka Creek (Intermittent)*			
	df	*F*	*P*		*df*	*F*	*P*
Season	1	1.99	0.140	Season	1	8.21	< **0.001**
Year	1	2.14	0.155	Year	1	1.07	0.406
Residual	18			Residual	18		

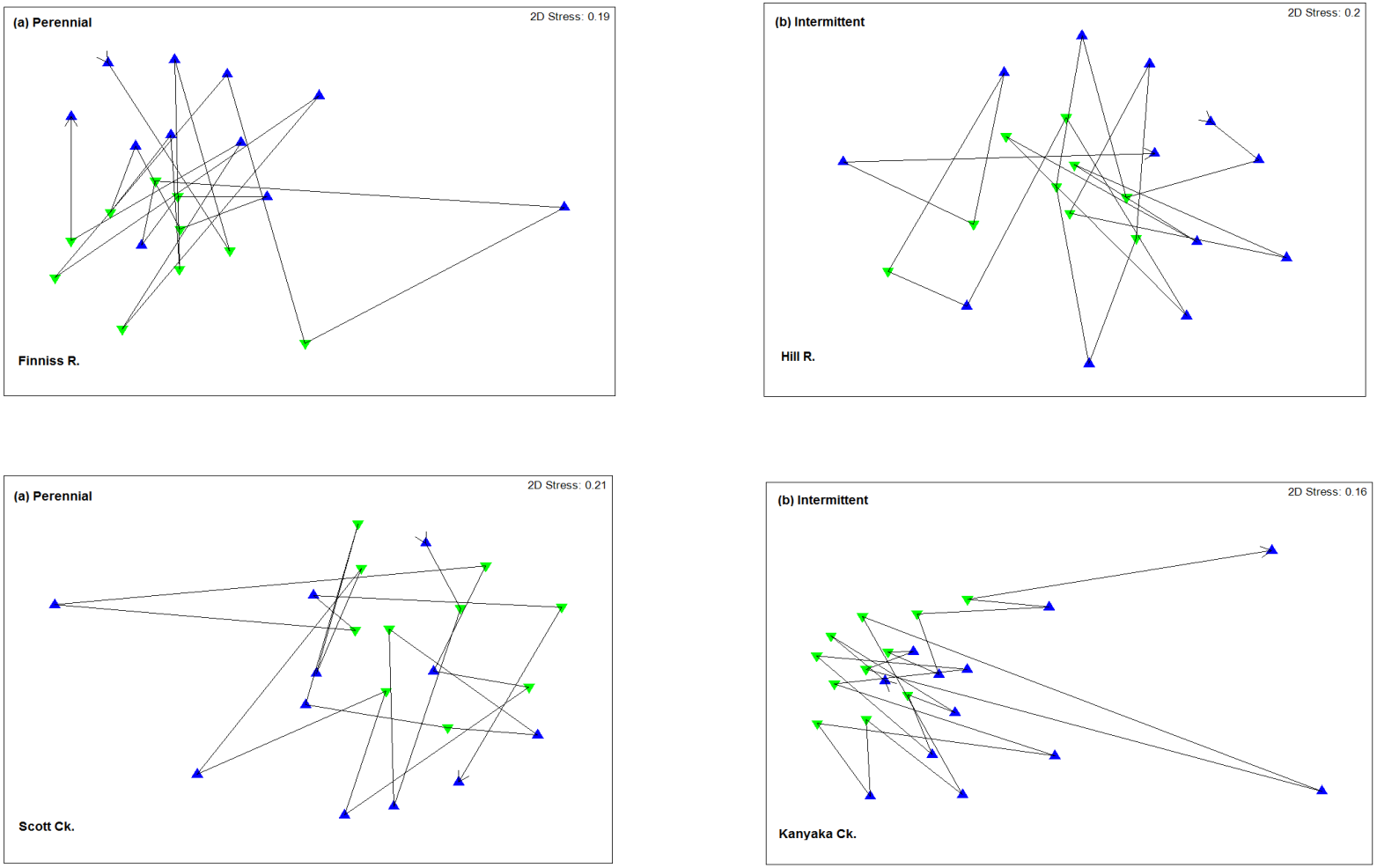

Fig. 1. Unconstrained ordination plots of taxonomic composition (individual sites across years and between seasons) based on Bray-Curtis similarity for a) perennial and b) intermittent sites. Symbols with ▼ represent autumn and ▲ represent spring. Sites names with "Ck" represents creek and "R" represent rivers. The lines connecting the dots represent trajectories of community structure across the years. Inverted arrow symbol indicate the start of the trajectory and the arrow head indicates the end of the trajectory.

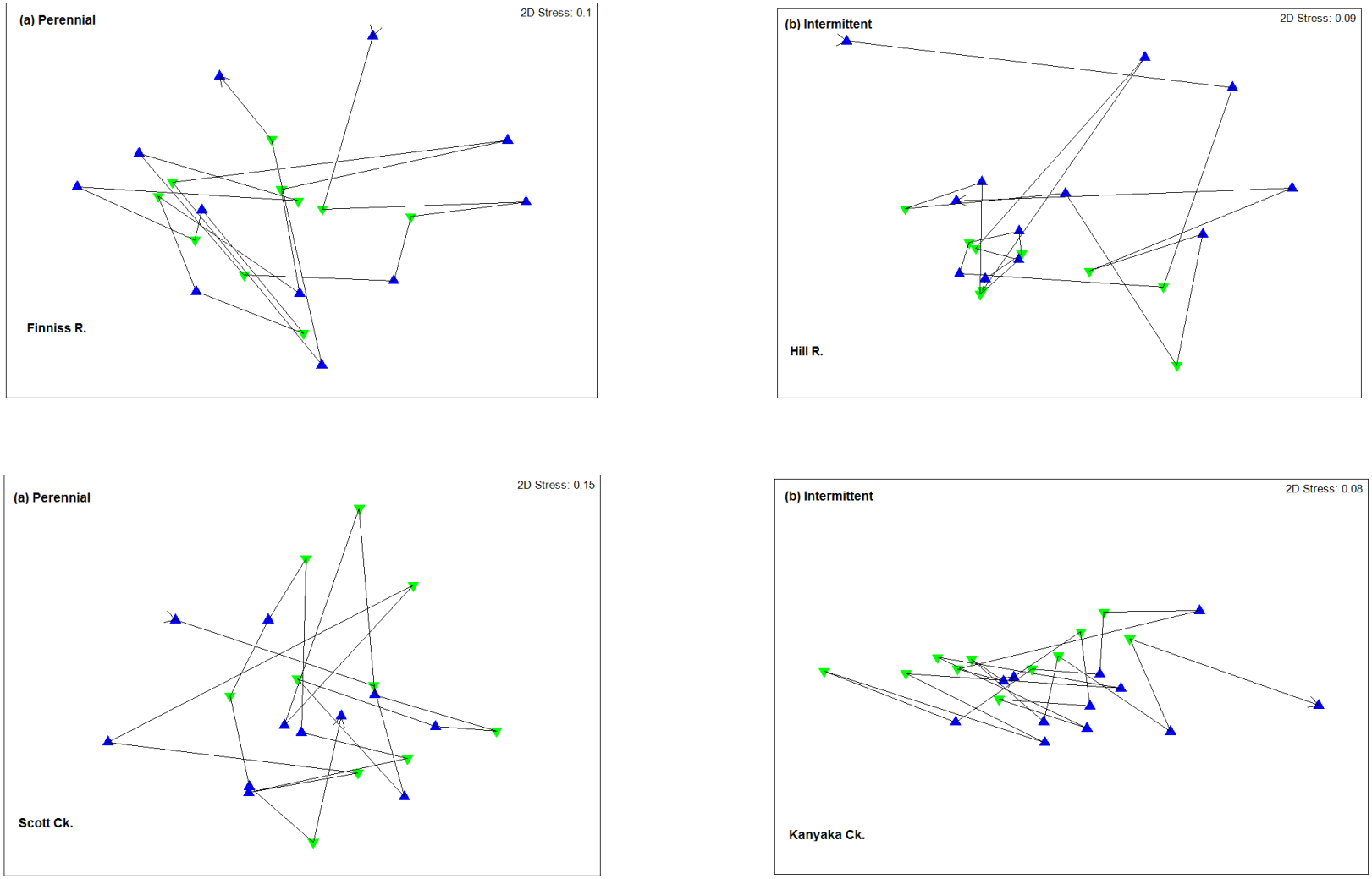

Fig. 2. Unconstrained ordination plots of trait composition (individual sites across years and between seasons) based on Gower similarity for a) perennial and b) intermittent sites. Symbols with ▼ represent autumn and ▲ represent spring. Sites names with "Ck" represents creek and "R" represent rivers. The lines connecting the dots represent trajectories of community structure across the years. Inverted arrow symbol indicate the start of the trajectory and the arrow head indicates the end of the trajectory.

5.4.2 Relationship of assemblage and trait composition to flow and salinity

Contrasting the taxonomic and trait-based approaches revealed that there was higher model uncertainty for the taxonomic-based models than for those based on traits (Tables 2A and 2B, Supporting Table S6). The top-ranked models predicting invertebrate structure overall explained more variation in trait space than taxa; in some instances a lot better (40%), and others, small gains (2%), although there was no discernible pattern related to whether or not the stream was perennial (Fig. 3). Interestingly, results from the BVSTEP procedure in PRIMER revealed that the differences in variances explained among streams were associated with differences in the number of different taxa in the most important trait categories (Table 3). Generally, the significant trait categories in Finniss and Hill River contained more taxa per trait than Scott and Kanyaka Creek (Table 3). Additionally, although slightly less distinct, Finniss and Hill River contained fewer dominant taxa (i.e. have lower values for Simpson's dominance index) for each of the most important traits than Scott or Kanyaka Creeks (Table 3).

For the taxonomic composition, salinity (in Finniss River) and flow permanence (in Scott Creek) were most strongly predictive of community structure in the perennial sites, explaining 6.3% and 8.2% of the total variation (Table 2A). In intermittent streams, the number of days since low flow event (in Hill River) and salinity (in Kanyaka Creek) were most strongly predictive, explaining 8.7% and 10.6 % of the total variation respectively (Table 2A). With the trait composition, a combination of the magnitude (Q75_30) and duration (DSHE) of high flows (in Finniss River), and salinity (in Scott Creek) were most strongly related to trait composition in the perennial sites, explaining 46.1% of the total variation in Finniss River, and 9.7% in Scott Creek (Table 2B). Intermittent sites were mostly affected by a combination of Q75_30 and salinity (in Hill River) and salinity (in Kanyaka Creek), explaining 27.6% and 13.5% of the variation in trait space respectively (Table 2B).

Table 2A. Model selection on DistLM shows predictors of taxonomic composition in perennial and intermittent sites. Abbreviations represent: AICc (Akaike Information Criterion), Δ AICc (delta AICc), R^2 (variance explained in taxonomic space), ID (model identification number), "Ta" (taxonomic composition) and "w" (model weight). Abbreviations for predictor variables are listed in Supporting Table S3 (Cond: salinity; Q75_30: high flow event > 75th percentile in the previous 30 days; flowPerm: flow permanence; DSLE: number of days since last low flow event; DSHE: number of days since last high flow event; and medQ: median flow). Bold text and numbers represent the best model for that particular site.

Model	ID	AICc	ΔAICc	R^2 (%)	w	Model	ID	AICc	ΔAICc	R^2 (%)	w
Finniss River (Perennial)						**Scott Creek (Perennial)**					
Ta ~ Cond	1	**142.52**	**0**	**6.29**	**0.084**	**Ta ~ flowPerm**	1	**144.94**	**0**	**8.18**	**0.074**
Ta ~ flowPerm	2	142.64	0.12	5.76	0.079	Ta ~ DSLE + flowPerm	2	144.95	0.01	8.16	0.074
Ta ~ medQ	3	142.65	0.13	5.70	0.079	Ta ~ Cond	3	145.24	0.30	6.87	0.064
Ta ~ Q75_30	4	142.81	0.29	4.98	0.073	Ta ~ Cond + flowPerm	4	145.24	0.30	6.87	0.064
Ta ~ DSLE	5	142.84	0.32	4.87	0.072	Ta ~ medQ	5	145.37	0.43	6.29	0.060
Ta ~ DSHE	6	142.85	0.33	4.82	0.071	Ta ~ DSHE	6	145.37	0.43	6.27	0.060
Ta ~ medQ + DSHE	7	143.88	1.36	12.29	0.043	Ta ~ medQ + flowPerm	7	145.37	0.43	6.29	0.060
Ta ~ Cond + flowPerm	8	143.94	1.42	12.04	0.041	Ta ~ DSHE + flowPerm	8	145.37	0.43	6.27	0.060
Ta ~ Cond + medQ	9	143.99	1.47	11.82	0.040	Ta ~ Q75_30	9	145.57	0.63	5.40	0.054
Ta ~ Cond + DSHE	10	144.15	1.63	11.15	0.037	Ta ~ Q75_30 + flowPerm	10	145.57	0.63	5.40	0.054
Ta ~ Cond + DSLE	11	144.15	1.63	11.17	0.037	Ta ~ Cond + DSLE	11	145.81	0.87	16.02	0.048
Ta ~ DSHE + flowPerm	12	144.15	1.63	11.13	0.037	Ta ~ Cond + Q75_30	12	146.07	1.13	14.97	0.042
Ta ~ Cond + Q75_30	13	144.17	1.65	11.06	0.037	Ta ~ DSHE + DSLE	13	146.23	1.29	14.34	0.039
Ta ~ medQ + flowPerm	14	144.23	1.71	10.82	0.036	Ta ~ Q75_30 + DSHE	14	146.34	1.40	13.88	0.037
Ta ~ medQ + Q75_30	15	144.24	1.72	10.76	0.036	Ta ~ medQ + DSLE	15	146.39	1.45	13.67	0.036
Ta ~ medQ + DSLE	16	144.26	1.74	10.68	0.035	Ta ~ Q75_30 + DSLE	16	146.43	1.49	13.52	0.035
Ta ~ Q75_30 + flowPerm	17	144.26	1.74	10.69	0.035	Ta ~ medQ + DSHE	17	146.61	1.67	12.77	0.032
Ta ~ DSLE + flowPerm	18	144.27	1.75	10.63	0.035	Ta ~ Cond + medQ	18	146.68	1.74	12.48	0.031
Ta ~ Q75_30 + DSHE	19	144.46	1.94	9.81	0.032	Ta ~ DSLE	19	146.73	1.79	0.00	0.030
Ta ~ DSHE + DSLE	20	144.55	2.03	9.44	0.030	Ta ~ Cond + DSHE	20	147.07	2.13	10.84	0.025
Ta ~ Q75_30 + DSLE	21	144.63	2.11	9.10	0.029	Ta ~ medQ + Q75_30	21	147.25	2.31	10.08	0.023
Ta ~ Cond + medQ + Q75_30 + DSHE + DSLE + flowPerm	22	154.08	11.56	30.90	0.000	Ta ~ Cond + medQ + Q75_30 + DSHE + DSLE + flowPerm	22	152.09	7.15	31.59	0.002
Hill River (Intermittent)						**Kanyaka Creek (Intermittent)**					
Ta ~ DSLE	1	**155.28**	**0**	**8.68**	**0.102**	**Cond**	1	**162.92**	**0**	**10.55**	**0.102**
Ta ~ Q75_30	2	155.38	0.10	8.22	0.097	Ta ~ Q75_30	2	163.21	0.29	9.41	0.088
Ta ~ flowPerm	3	155.76	0.48	6.56	0.080	Ta ~ Q75_30 + DSLE	3	163.59	0.67	17.99	0.073
Ta ~ medQ	4	156.38	1.10	3.75	0.059	Ta ~ flowPerm	4	163.65	0.73	7.68	0.071

Ta ~ DSLE + flowPerm	5	156.47	1.19	15.19	0.056	Ta ~ medQ + DSLE	5	163.78	0.86	17.32	0.066
Ta ~ Cond	6	156.51	1.23	3.18	0.055	Ta ~ DSLE	6	163.92	1.00	6.60	0.062
Ta ~ DSHE	7	156.53	1.25	3.08	0.054	Ta ~ medQ + Q75_30	7	164.14	1.22	16.01	0.055
Ta ~ Q75_30 + flowPerm	8	156.54	1.26	14.90	0.054	Ta ~ medQ	8	164.6	1.68	3.80	0.044
Ta ~ Q75_30 + DSHE	9	156.81	1.53	13.81	0.047	Ta ~ Cond + medQ	9	164.69	1.77	13.99	0.042
Ta ~ DSHE + DSLE	10	157.03	1.75	12.90	0.042	Ta ~ medQ + flowPerm	10	164.73	1.81	13.84	0.041
Ta ~ Cond + DSLE	11	157.09	1.81	12.66	0.041	Ta ~ DSHE	11	164.75	1.83	3.17	0.041
Ta ~ Q75_30 + DSLE	12	157.16	1.88	12.35	0.040	Ta ~ medQ + DSHE	12	164.76	1.84	13.72	0.041
Ta ~ medQ + DSLE	13	157.17	1.89	12.33	0.040	Ta ~ DSLE + flowPerm	13	164.76	1.84	13.72	0.041
Ta ~ Cond + Q75_30	14	157.25	1.97	12.00	0.038	Ta ~ Q75_30 + flowPerm	14	164.84	1.92	13.42	0.039
Ta ~ medQ + Q75_30	15	157.26	1.98	11.95	0.038	Ta ~ Q75_30 + DSHE	15	164.98	2.06	12.90	0.036
Ta ~ medQ + flowPerm	16	157.49	2.21	10.99	0.034	Ta ~ Cond + Q75_30	16	165.05	2.13	12.62	0.035
Ta ~ Cond + flowPerm	17	157.64	2.36	10.35	0.031	Ta ~ DSHE + flowPerm	17	165.48	2.56	10.97	0.028
Ta ~ DSHE + flowPerm	18	157.72	2.44	10.00	0.030	Ta ~ Cond + flowPerm	18	165.5	2.58	10.88	0.028
Ta ~ medQ + DSHE	19	158.41	3.13	6.99	0.021	Ta ~ Cond + DSLE	19	165.81	2.89	9.69	0.024
Ta ~ Cond + DSHE	20	158.46	3.18	6.79	0.021	Ta ~ DSHE + DSLE	20	165.81	2.89	9.67	0.024
Ta ~ Cond + medQ	21	158.59	3.31	6.18	0.019	Ta ~ Cond + DSHE	21	166.51	3.59	6.87	0.017
Ta ~ Cond + medQ + Q75_30 + DSHE + DSLE + flowPerm	22	167.55	12.27	30.32	0.000	Ta ~ Cond + medQ + Q75_30 + DSHE + DSLE + flowPerm	22	172.42	9.5	35.07	0.001

Table 2B. Model selection on DistLM shows predictors of trait composition in perennial and intermittent sites. Abbreviations represent: AICc (Akaike Information Criterion), Δ AICc (delta AICc), R^2 (variance explained in taxonomic space), ID (model identification number), "Tr" (trait composition) and "w" (model weight). Abbreviations for predictor variables are listed in Supporting Table S3 (Cond: salinity; Q75_30: high flow event > 75th percentile in the previous 30 days; flowPerm: flow permanence; DSLE: number of days since last low flow event; DSHE: number of days since last high flow event; and medQ: median flow)

Model	ID	AICc	ΔAICc	R^2 (%)	w	Model	ID	AICc	ΔAICc	R^2 (%)	w
Finniss River (Perennial)						**Scott Creek (Perennial)**					
Tr ~ Q75_30 + DSHE	1	**-123.16**	**0**	**46.10**	**0.262**	**Tr ~ Cond**	1	**-98.543**	**0**	**9.68**	**0.099**
Tr ~ Q75_30	2	-122.56	0.6	36.80	0.194	Tr ~ Cond + flowPerm	2	-98.540	0.003	9.67	0.099
Tr ~ medQ + Q75_30	3	-122.34	0.82	43.96	0.174	Tr ~ Q75_30	3	-98.15	0.39	7.98	0.081
Tr ~ Q75_30 + flowPerm	4	-121.77	1.39	42.41	0.131	Tr ~ Q75_30 + flowPerm	4	-98.15	0.39	7.98	0.081
Tr ~ Q75_30 + DSLE	5	-121.76	1.40	42.38	0.130	Tr ~ Cond + DSLE	5	-97.922	0.62	18.38	0.073
Tr ~ Cond + Q75_30	6	-119.5	3.66	35.85	0.042	Tr ~ DSLE	6	-97.824	0.72	6.54	0.069
Tr ~ DSHE	7	-117.29	5.87	18.75	0.014	Tr ~ DSLE + flowPerm	7	-97.824	0.72	6.54	0.069
Tr ~ Cond + DSHE	8	-116.37	6.79	25.51	0.009	Tr ~ Q75_30 + DSLE	8	-97.654	0.89	17.33	0.064
Tr ~ DSHE + flowPerm	9	-116.12	7.04	24.63	0.008	Tr ~ DSHE	9	-96.977	1.57	2.69	0.045
Tr ~ medQ + DSHE	10	-115.53	7.63	22.50	0.006	Tr ~ DSHE + flowPerm	10	-96.977	1.57	2.69	0.045
Tr ~ DSHE + DSLE	11	-115.23	7.93	21.38	0.005	Tr ~ Cond	11	-96.404	2.14	0.00	0.034
Tr ~ medQ	12	-114.71	8.45	8.12	0.004	Tr ~ medQ	12	-96.399	2.14	0.02	0.034
Tr ~ Cond	13	-114.46	8.7	7.02	0.003	Tr ~ medQ + flowPerm	13	-96.399	2.14	0.02	0.034
Tr ~ flowPerm	14	-114.13	9.03	5.56	0.003	Tr ~ Cond + Q75_30	14	-96.269	2.27	11.69	0.032
Tr ~ DSLE	15	-114.11	9.05	5.49	0.003	Tr ~ Cond + medQ	15	-95.789	2.75	9.65	0.025
Tr ~ Cond + medQ + Q75_30 + DSHE + DSLE + flowPerm	16	-113.47	9.69	58.54	0.002	Tr ~ DSHE + DSLE	16	-95.649	2.89	9.04	0.023
Tr ~ Cond + medQ	17	-113.31	9.85	13.83	0.002	Tr ~ medQ + DSLE	17	-95.515	3.03	8.46	0.022
Tr ~ medQ + flowPerm	18	-113.18	9.98	13.31	0.002	Tr ~ medQ + Q75_30	18	-95.41	3.13	8.00	0.021
Tr ~ Cond + flowPerm	19	-113	10.16	12.57	0.002	Tr ~ Cond + DSHE	19	-95.329	3.21	7.65	0.020
Tr ~ Cond + DSLE	20	-112.99	10.17	12.52	0.002	Tr ~ Q75_30 + DSHE	20	-95.053	3.49	6.43	0.017
Tr ~ medQ + DSLE	21	-112.83	10.33	11.86	0.001	Tr ~ medQ + DSHE	21	-94.179	4.36	2.45	0.011
Tr ~ DSLE + flowPerm	22	-112.63	10.53	10.99	0.001	Tr ~ Cond + medQ + Q75_30 + DSHE + DSLE + flowPerm	22	-87.897	10.65	20.54	0.000
Hill River (Intermittent)						**Kanyaka Creek (Intermittent)**					
Tr ~ Cond + Q75_30	1	**-68.573**	**0**	**27.63**	**0.161**	**Tr ~ Cond**	1	**-91.412**	**0**	**13.54**	**0.134**
Tr ~ Q75_30 + DSLE	2	-67.816	0.76	24.97	0.110	Tr ~ medQ	2	-90.766	0.65	7.22	0.097

Tr ~ medQ + Q75_30	3	-66.923	1.65	21.71	0.071	Tr ~ Cond + medQ	3	-90.533	0.88	16.52	0.086
Tr ~ Q75_30	4	-66.896	1.68	10.66	0.070	Tr ~ flowPerm	4	-90.151	1.26	4.70	0.071
Tr ~ DSLE	5	-66.645	1.93	9.59	0.061	Tr ~ medQ + DSLE	5	-90.132	1.28	15.05	0.070
Tr ~ medQ	6	-66.586	1.99	9.33	0.060	Tr ~ Cond + DSLE	6	-89.717	1.70	13.50	0.057
Tr ~ Cond + DSLE	7	-66.333	2.24	19.48	0.053	Tr ~ Cond + DSHE	7	-89.513	1.90	12.74	0.052
Tr ~ medQ + DSLE	8	-66.269	2.30	19.23	0.051	Tr ~ DSHE	8	-89.437	1.98	1.70	0.050
Tr ~ flowPerm	9	-65.993	2.58	6.74	0.044	Tr ~ medQ + flowPerm	9	-89.334	2.08	12.05	0.047
Tr ~ Q75_30 + flowPerm	10	-65.896	2.68	17.79	0.042	Tr ~ Cond + flowPerm	10	-89.266	2.15	11.79	0.046
Tr ~ DSLE + flowPerm	11	-65.493	3.08	16.19	0.035	Tr ~ Cond + Q75_30	11	-89.026	2.39	10.87	0.040
Tr ~ Cond	12	-65.43	3.14	4.20	0.033	Tr ~ Q75_30	12	-88.801	2.61	1.06	0.036
Tr ~ medQ + flowPerm	13	-65.386	3.19	15.77	0.033	Tr ~ DSLE	13	-88.715	2.70	1.44	0.035
Tr ~ DSHE	14	-65.103	3.47	2.70	0.028	Tr ~ Q75_30 + DSLE	14	-88.676	2.74	9.50	0.034
Tr ~ DSHE + flowPerm	15	-64.84	3.73	13.55	0.025	Tr ~ medQ + DSHE	15	-88.526	2.89	8.91	0.032
Tr ~ medQ + DSHE	16	-64.802	3.77	13.39	0.024	Tr ~ DSLE + flowPerm	16	-88.134	3.28	7.34	0.026
Tr ~ DSHE + DSLE	17	-64.795	3.78	13.36	0.024	Tr ~ medQ + Q75_30	17	-87.953	3.46	6.61	0.024
Tr ~ Q75_30 + DSHE	18	-64.52	4.05	12.22	0.021	Tr ~ DSHE + flowPerm	18	-87.785	3.63	5.93	0.022
Tr ~ Cond + flowPerm	19	-64.218	4.36	10.95	0.018	Tr ~ Q75_30 + DSHE	19	-87.272	4.14	3.80	0.017
Tr ~ Cond + medQ	20	-64.052	4.52	10.24	0.017	Tr ~ Q75_30 + flowPerm	20	-86.672	4.74	1.26	0.012
Tr ~ Cond + DSHE	21	-64.035	4.54	10.17	0.017	Tr ~ DSHE + DSLE	21	-86.59	4.82	0.91	0.012
	22					Tr ~ Cond + medQ +	22				
Tr ~ Cond + medQ + Q75_30 + DSHE + DSLE + flowPerm		-58.902	9.67	44.39	0.001	Q75_30 + DSHE + DSLE + flowPerm		-82.024	9.39	34.83	0.001

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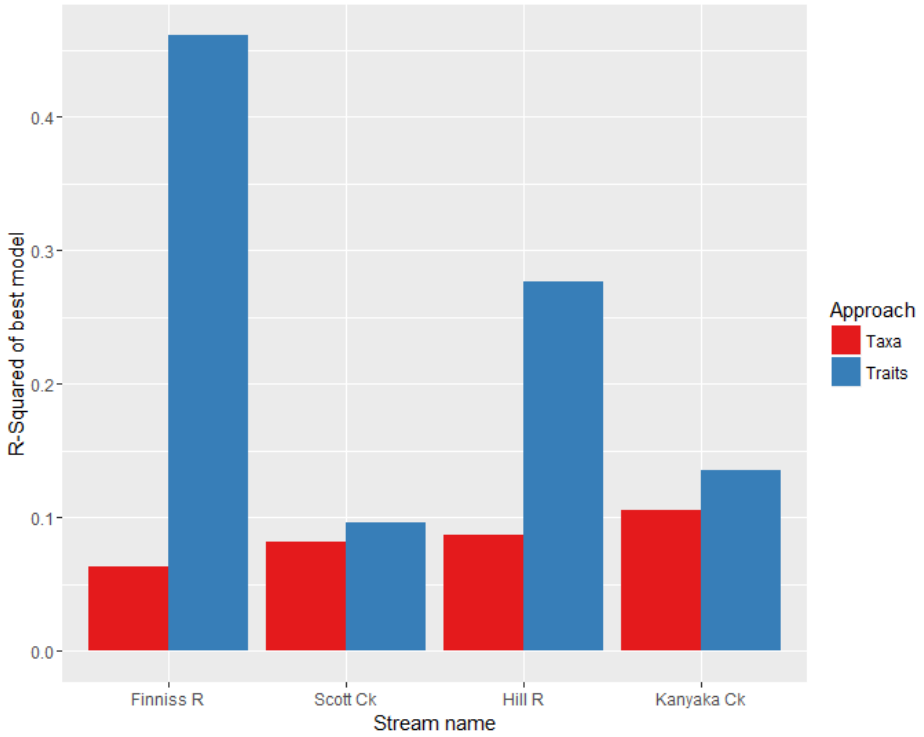

Fig 3. Bar plot of the R^2 (variance explained) for the best model predicting macroinvertebrate taxonomic or trait composition in perennial and intermittent sites. "R" denotes "River" and "Ck" denotes "Creek". Perennial sites are Finniss River and Scott Creek, while intermittent sites are Hill River and Kanyaka Creek.

Table 3: Number of macroinvertebrate families and dominance associated with the most important trait categories from the best model for each site. The most important trait categories and the number of their associated taxa were those identified by the BVSTEP procedure. Where the difference in variance explained between best taxonomic and best trait based models was greater (Finniss River, Hill River), there was a general pattern for greater numbers of macroinvertebrate families and lower taxonomic dominance per trait, than for sites where the difference in models was smaller (Scott Creek, Kanyaka Creek). Abbreviations for trait categories are listed in Supporting Table S1.

Trait category	Number of different taxa associated with trait category	Simpson's dominance (*D*)
Finniss River (perennial; R^2 difference between best models = 40%)		
Volt2	63	1.989
Habi5	41	3.250
Resp1	59	2.428
Exit2	70	2.837
Sync1	59	2.249
Scott Creek (perennial; R^2 difference between best models = 2%)		
Flgt1	19	7.165
Mat2	22	6.928
Arm3	28	7.241
Hill River (intermittent; R^2 difference between best models = 19%)		
Crwl2	26	5.125
Swim2	45	2.250
Exit2	70	2.837
Kanyaka Creek (intermittent; R^2 difference between best models = 3%)		
Habi2	18	6.305
Habi5	41	3.250
Crwl2	26	5.125

5.5 Discussion

Here, we examined within-site temporal patterns in taxonomic and trait composition to assess whether traits performed consistently better than taxonomy. We found that flow and salinity variables explained more of the variation in trait composition than taxa, but the gains in variance explained varied greatly, ranging from 3% in Kanyaka Creek to 40% in Finniss River. As predicted, biological trait composition was more similar between seasons than taxonomic composition, and this accords with findings from previous studies (Bêche and Resh, 2007, Milner et al., 2006). Thus, there is more taxonomic turnover, but trait composition in pools remains fairly constant (Snook and Milner, 2002). This suggests that biomonitoring based on traits could be reduced to one sampling event per year. Further, intermittent streams were less similar between seasons than perennial streams, which also conformed to our predictions.

5.5.1 Temporal change in assemblage and trait composition

The general consensus view in the literature is that biological trait composition should be more similar between seasons and years than taxonomic composition (Bêche et al., 2006, Milner et al., 2006, Southwood, 1988, Southwood, 1977). By contrast, the occurrence and abundance of individual taxa varies from year-to-year, leading to less predictable taxonomic composition (Bêche and Resh, 2007, Townsend and Hildrew, 1994). In our study, we found that, within sites, biological trait composition was more similar between seasons than taxonomic composition, which accords with findings from other studies (Bêche and Resh, 2007, Milner et al., 2006). The relative similarity of biological traits between seasons may imply that some traits are equally advantageous or useful in both seasons.

Our study also showed that, at the intermittent sites, species with traits that differed between seasons had life histories (e.g. aerial respiration, skating, faster maturation, very short life cycle) that may have allowed them to survive drying and elevated salinity during the dry period (i.e. autumn in our study; resistant traits). In contrast, traits that differed between seasons in perennial streams included gill respiration, flattened body shape, high rheophily and large body size. These traits are characteristic of flowing conditions (e.g. high flows, lower temperatures and high dissolved oxygen content), and regardless of seasons, perennial streams usually maintain flows throughout the year. Thus, both intermittent and perennial streams harbour traits that enable them to be resilient or resistant to different phases of environmental conditions in intermittent (characterised by some dry periods) and perennial (flows throughout the year) streams.

Further, our study revealed that both taxonomic and trait composition were less similar between seasons in the intermittent than in the perennial sites. That is, there is more turnover in intermittent streams (average dissimilarity between seasons ranged from 16 to 55%) than perennial streams (dissimilarity ranged from 11 to 43%). This pattern may be expected because intermittent streams are more likely to lose species by chance during dry periods (Brooks and Haeusler, 2016, Lake, 2003) than perennial streams. Our results are in accordance with i Caparrós (2003) in Spain who also found that intermittent sites exhibited more variation in trait and taxonomic composition than perennial streams.

Lastly, our study revealed that neither trait nor taxonomic composition showed marked directional changes through time, despite the significant interannual variability in

taxonomic composition over the study period. This may be because the macroinvertebrate communities were tolerant of environmental extremes (e.g. high salinity and low flows), and had life history traits (e.g. multivoltinism, strong flying ability) that facilitated population recovery (Charvet et al., 2000).

5.5.2 Relationship of assemblage and trait composition to flow and salinity

An important question in community ecology, which our study sought to address, is whether functional traits convey more or different information about ecological communities than focussing on the taxa. However, results from freshwater systems have been variable in this regard: functional traits may (Hoeinghaus et al., 2007, Reynaga and Dos Santos, 2013), or may not (Finn and Poff, 2005) provide more information than taxonomic structure about community patterns and community-environment relationships. Our findings revealed that, generally, trait analysis were better explained by flow and salinity variables than taxonomic analysis, with models performing a lot better in some cases (e.g. explains 40% more of variance in trait space than taxa in Finniss River) and less so in other cases (e.g. explains 3% more of the variance in trait space than taxa in Kanyaka Creek). However, the performance of the models had no discernible pattern related to whether or not the stream was perennial.

It was surprising that the variance explained by the best models, especially, in trait composition, varied greatly between individual sites of the same stream type (i.e. perennial or intermittent stream). We found that differences in variance explained was associated with the number of taxa and the pattern of dominance of taxa sharing the same trait. Generally, where the difference in variance explained was greater, there were more taxa per trait and the dominance of taxonomic families was smaller than where the difference in variance explained was small. Thus, where there are more taxa sharing the same trait category and sharing it more evenly, then traits will likely reduce the redundancy associated with using taxonomic identities, so that the trait-space has greater explanatory power than the taxonomic space. Further, the best model explained 46.1% of the variation in trait composition (Fig. 3), which is broadly similar to the variance explained (10 – 48%) in the wider literature (e.g. Barrios, 2015, Heino et al., 2007b, Horrigan and Baird, 2008, Ieromina et al., 2016). However, there is still > 50% of unexplained variation in our study, which suggests that, perhaps, the variables we measured did not fully describe the circumstances that are important for the macroinvertebrate traits.

5.6 Conclusion

Our study addressed the question of whether traits would perform substantially better than taxa, when analyses focussed within sites. We demonstrated that functional traits performed better as indicators of environmental conditions than focussing on taxa. However, it should be noted that the gains in variance explained varied greatly, with models performing a lot better in some cases and less so in other cases, and this was associated with the number and dominance of taxa per trait. Further, our study showed that macroinvertebrate trait composition was more similar between seasons than taxonomic composition, suggesting that some traits are equally advantageous in both the dry and wet seasons. Thus, a management outcome of this study is that biomonitoring based on traits could be reduced to one sampling event per year provided that the aims of the monitoring program were well-served by using trait space. For instance, if management aim is to detect environmental impacts, or characterize functional composition, and traits are saying the same thing about these aspects between seasons, then reducing sampling to one event per year could work.

Chapter 6

General discussion

I evaluated trait-based and taxonomic-based approaches used to assess environmental conditions in stream ecosystems by using a large dataset from South Australia derived from the Australian Rivers Assessment System (AUSRIVAS) (Davies, 2000). To accomplish this, I first undertook a traditional taxonomic-based study, where I related land-use, geographic and environmental variables to community composition in order to determine the most influential physical variables structuring communities. From this study, I concluded that physical variables explained a small proportion of variation in taxonomic space (23.7% and 27.3% in autumn and spring respectively), with salinity being the key environmental driver, in addition to known importance of flow. The low explained variance was broadly consistent with the wider literature. There were also substantial biogeographic effects on the taxonomic composition. Subsequently, I focussed on salinity and flow as predictors of macroinvertebrate community structure.

I then systematically reviewed the trait-based literature of lotic macroinvertebrate responses to effects of flow and salinity to better understand the temporal and geographic extent of previous studies, methodological approaches used to relate flow and salinity to macroinvertebrate traits, and examine the extent to which there have been consistent outcomes across studies. This showed that, studies were accumulating at a slow and steady rate and that there were broad trends in which traits appear to be associated with flow and salinity. In terms of geographic coverage, studies were strongly biased towards Western Europe and North America. There were also inconsistencies in some trait responses to effects of these stressors which may be due to differences in methodological approaches among studies. I did not find any studies that examined interactions between effects of flow and salinity on macroinvertebrate traits, even though high salinities and low flows often co-occur and may interact, especially in temporary streams. Thus, I hypothesized that interactions between flow and salinity may underlie some inconsistently behaving traits in the literature.

Consequently, I investigated the simultaneous effects of flow and salinity on macroinvertebrate traits to examine the extent to which their individual and interactive effects

were important. I found that traits (e.g. burrowing, tegument respiration and collector-gathering) with inconsistent behaviour in the literature were indeed associated with interactions between flow and salinity. The interactions appeared to be driven by the differential responses of different taxa with the same trait. Further, flow and salinity variables explained more of the variation in trait composition (27.3% in autumn, 36.7% in spring) than taxa (20.9% in autumn, 27.7% in spring). However, there were still strong biogeographic effects, even for trait composition.

To evaluate traits in comparison to taxa in more detail, I analysed within-site data sets. By focussing within sites, I was able to overcome biogeographic signals that affect taxonomic and trait composition, and was able to determine whether models better predict traits than taxa. I analysed data separately within each of four sites with the most complete flow and environmental records, two each from perennial and intermittent flow regimes. I predicted that trait composition would be more similar between seasons than taxa because there is more turnover with taxa over time than with traits. I also predicted that in both taxonomic and trait composition, intermittent streams would be less similar between seasons than perennial streams, because intermittent streams are more likely to lose species by chance during dry periods. These predictions were generally supported by the data. Further, trait analyses were better explained by flow and salinity variables than taxonomic analyses, although the improvements ranged from 2% to 40% of additional variance explained. However, there was no consistent pattern corresponding to flow regime. The differences in variance explained were associated with the number of taxa and the pattern of dominance of taxa sharing the same trait. Generally, where the difference in variance explained was greater, there were more taxa per trait and the dominance of taxonomic families was smaller than where the difference in variance explained was small.

My study has shown that the use of functional traits performed better as indicators of environmental conditions than focussing on taxa, although the gains in variance explained varied greatly, and the use of traits did not overcome biogeographic effects. I also identify a range of other challenges associated with the use of traits namely: the taxonomic resolution of the taxa characterised by traits (family, genus, species or mixed), the number and type of traits being considered, trait data conversion (quality), correlation among traits, how trait-environment relationship are quantified (Heino et al., 2013), and phylogenetic relatedness

among traits. In the subsequent sections, I discuss some challenges associated with using traits, in the light of the results of my thesis.

6.1 Challenges

6.1.1 Traits and biogeography

My study showed that sites spanning multiple catchments were a major part of the variation in my analysis, suggesting that traits did not overcome biogeographic effects as theory predicts (McGill et al., 2006). This is in congruence with similar studies that observed that traits vary across rivers basins at multiple catchments for fish species in North America (Mims et al., 2010) and Europe (Logez et al., 2013), as well as for macroinvertebrate in Europe (Bhowmik and Schäfer, 2015). In contrast, Statzner et al. (2004) and White et al. (2016) reported that the trait composition of stream macroinvertebrates did not vary geographically across catchments in Europe. The differences in outcomes across these studies may be due to analytical and methodological differences and the spatial extent of the study. For instance, Statzner et al. (2004) analysed their ordinal and mixed trait data set using Euclidean distance, while White et al. (2016) used Bray-Curtis dissimilarities. However, Mims et al. (2010) and Logez et al. (2013) used an appropriate similarity measure such as Gower's similarity index, which has been advocated by Pavoine et al. (2009) and Schmera et al. (2015) to be used for ordinal and nominal trait data set. Thus, in order to establish whether convergence in macroinvertebrate trait patterns exist among biogeographic regions or spatial scales, further studies are warranted. Such studies should be based on using the same methodological approaches, conducted at different spatial scales and environmental settings, and in different organismal groups, such as stream macroinvertebrates and fish assemblages.

6.1.2 Taxonomic and trait resolution

I found that published trait data sets usually showed mixed levels of taxonomic resolution (Chapter 3), with coarser resolution for the more cryptic taxa such as the chironomids and oligochaetes. These groups are important in quantifying both diversity patterns and ecosystem processes in a variety of habitat types. However, logistical constraints and the need for high levels of taxonomic expertise often hinders identifications to finer levels for these frequently abundant taxonomic groups (Franquet, 1999). Thus, studies that are unable to identify these to lower taxonomic levels either use coarser taxonomy (e.g. Cid et al., 2016)

or exclude them from the analysis (e.g. Gallardo et al., 2009). However, assigning traits at coarse taxonomic resolution may obscure important responses, while excluding from the analysis the more cryptic taxonomic groups such as mites, chironomids and oligochaetes, could also restrict the suite of trait categories available for identifying the type and level of environmental impact (Culp et al., 2011).

Further, Serra et al. (2016) argued that assigning traits at species level for chironomids (at least) provides better detection of ecological patterns and perturbations than family or genus. If further studies reveal that assigning traits at finer taxonomic resolution for other groups (e.g. oligochaetes and mites) result in better detection of environmental effects, then improved taxonomic resolution will be needed. This will involve increased efforts, extensive taxonomic expertise, logistics and financial resources (Jones, 2008), especially in areas where regional taxonomic knowledge is limited. However, if genus or species level resolution is needed to assign traits, then the added costs of doing so mitigate any savings purportedly made by using traits in applied studies.

6.1.3 Trait measurement and representation

Researchers have used different methods to quantify traits, and this diversity of methods presents challenges for comparing trait studies. For instance, trait tables may be expressed on different measurement scales (e.g. nominal, ordinal, interval, ratio or quantitative variables) (Schmera et al., 2015). Ordinal and mixed data sets pose challenges for similarity-based analysis (Pavoine et al., 2009, Schmera et al., 2015), and can be overcome by using appropriate similarity measures for ordinal data such as Gower's similarity index (Anderson et al., 2006, Gower, 1971). However, these recommendations are sometimes not followed [e.g. Brooks et al. (2011), who analysed ordinal data using Euclidean metric]. Therefore it would be useful for traits to be measured and quantified consistently, and for a detailed definition of each trait and its related categories or modalities to be provided ideally with metadata detailing how the information was derived (Baird et al., 2011) in trait papers.

The number and types of taxonomic groups used in biomonitoring programmes may also affect biomonitoring outcomes. For effective incorporation of trait-based approaches into biomonitoring programmes, the effect of taxonomic bias towards a subset of taxa, especially for easily identifiable taxa or known sensitive taxa (e.g. Trichoptera, Plecoptera and Ephemeroptera) should be explored. This is because other taxonomic groups (e.g.

oligochaetes and chironomids) are equally important in quantifying both diversity patterns and ecosystem processes in a variety of habitat types. Excluding such taxa from trait-based analysis could restrict the suite of trait categories available for identifying the type and level of environmental impact (Culp et al., 2011).

6.1.4 Traits and phylogenetic relatedness

Traits may often be phylogenetically linked (trait "syndromes") (Bêche and Resh, 2007, Poff et al., 2006) and this may create redundancies that complicate interpretation (Poff et al., 2006, Poteat et al., 2015). This is because phylogenetically linked traits may respond similarly or in tandem to an environmental gradient (i.e. multiple traits may relate strongly to similar underlying environmental stressors without necessarily having a hypothesized link to the stressors). For example, Van Kleef et al. (2006) found that ability to fly was not important in the re-colonisation of restored habitats. This counterintuitive result arose because many species with active flight in their study were also carnivorous, and hence recruitment was delayed by scarcity of prey. As a possible solution to minimise the potential problem of trait syndromes, Poff et al. (2006), Horrigan and Baird (2008) and Chessman (2015) suggested using traits that may be phylogenetically independent (e.g. thermophily, rheophily, crawling abilities, among others). Furthermore, the challenges associated with trait syndromes may be minimized by selective choice of traits with a hypothesized mechanistic link with the stressors of interest (Culp et al., 2011, Rubach et al., 2011).

Another possible solution for evolutionary linkages among traits may be to see them as part of complex adaptations or combinations of co-evolved attributes based on known functional relationships (i.e. life-history strategies) among them (Verberk et al., 2008b). Thus, an approach where different species are assigned to the same life-history strategy, according to their ability to solve similar ecological problems may be useful (Verberk et al., 2008a). Alternatively, recognition of phylogenetically correlated traits may provide a way to derive information for poorly studied taxa, thereby allowing the identification of taxa that respond similarly to a stressor (Buchwalter et al., 2008).

6.2 Traits and understanding effects of salinity and flow

My study showed that, generally, high salinity promoted ovoviviparity, multivoltinism, fliers and aerial respiration, while gill respiration and taxa with medium-high physiological

sensitivity to salinity decreased. The ecological explanations supporting these trait relationships with increasing salinity are as follows. Ovoviviparity was favoured because it protects the young by isolating the eggs from external elevated salt concentrations (Piscart et al., 2006). Multivoltine life cycles are held to be advantageous since it increases the chances of survival of offspring (Kefford et al., 2004). Young individuals commonly have a lower salinity tolerance than older stages (Kefford et al., 2004), and are likely to grow more slowly in response to pulses of highly saline water, thus forcing adult females (e.g. Elmidae) produce more than two generations per year to increase chances of survival of their offspring (Canedo-Arguelles et al., 2012). Unsurprisingly, the number of both medium and highly salt-sensitive taxa decreased with increased salinity, as would be expected given the physiological basis of these two trait categories (Schäfer et al., 2011). Gill-respiring taxa also decreased in salty streams, most likely because such streams commonly have low dissolved oxygen concentrations which should favour macroinvertebrates with morphological structures (e.g. spiracles, siphons, plastrons) to breathe atmospheric oxygen (Chapman et al., 2004, Hinton, 1976).

Additionally, my study showed that, high rheophily, cool eurythermality and gill respiration decreased during low flows (which are typified by high water temperatures and low oxygen concentrations), while aerial respirers and strong fliers increased. Unsurprisingly, as flow permanence increased, high rheophily and cool eurythermality also increased. The warmer conditions that characterise low flows might have reduced the abundance of thermally sensitive taxa, while the cooler conditions when flow permanence increased could have allowed for such taxa to rebound (Chessman, 2015). Furthermore, reduced flow often leads to decreases in overall habitat availability and an increased proportion of pool habitats. These changes would favour taxa with the ability to move as habitat contracts, e.g., taxa with strong flying strength, high crawling rate or burrowers, and taxa adapted to the warmer temperatures and lower oxygen levels in pools (Walters, 2011). Additionally, traits characteristic of slow maturation (holometaboly and univoltinism) were favoured during increased flow permanence. This is because invertebrates with slower maturation may not recruit more successfully during low flows as they may not be able to complete development from egg to adult during brief flow episodes or in residual pools. During favourable environmental conditions (e.g. high flows), invertebrates may tend to invest in fewer but larger eggs to improve fitness of their offspring, and this trade-off has been reported by

Berrigan (1991). It was therefore not surprising that we found an increase in univoltinism (only one brood or generation per year) during high flows in our study.

However, from my study, it should be noted that some traits (e.g. burrowing, tegument respiration and collector-gathering) are rather indicative of interactive effects between flow and salinity, and that these traits may not be accurate indicators of salinization alone as they also responded to effects of flow intermittency. For example, ovoviviparity may be an accurate indicator of effects of salinity, as this trait responded to only salinity (and not flow) effects in my study, as was also reported by Díaz et al. (2008), Piscart et al. (2006), Szöcs et al. (2014).

6.3 Trait database

To achieve the operational implementation of using biological traits as indicators of stream conditions, one major obstacle has to be cleared: the creation of trait databases. While the trait database I compiled (see methods section of Chapter 4 for details) is strongly focussed on detecting effects of salinity and flow alterations, however, there is scope to extend beyond my existing database by adding traits that are adapted to revealing impacts of the multitude of potential anthropogenic stressors (e.g. nutrients and sedimentation) in stream ecosystems. For this purpose, some conventions are required, so that the traits of stream invertebrates are consistently defined and described across large spatial scales. Further, more effort and logistical support is required to fill the gaps in the information for some poorly studied but abundant taxonomic groups (e.g. most dipterans, oligochaetes) to enable allocation of traits at species level for these groups in my database. This is very important, especially in the light of research by Serra et al. (2016) and King and Richardson (2002) who argued that allocating traits at genus or species level for some groups (e.g. chironomids) have the greatest potential to improve the signals provided by ecological assessment tools.

6.4 Implications for applied management

By undertaking a traditional taxonomic study and relating land-use, geographic and environmental variables to community composition, I found that salinity was a key environmental driver, in addition to the known importance of flow. Thus, maintaining temporary streams will require improved catchment management aimed at sustaining

seasonal flows and critical refuge habitats, while also limiting the damaging effects from increased salinity, agriculture and urban developments.

By focussing on within-site analyses and contrasting taxonomic with trait-based approaches (Chapter 5), I found that trait composition was more similar between seasons than taxonomic composition because there was more turnover in taxa than trait structure. This suggests that some traits are equally advantageous in both the dry and wet seasons. Thus, a management outcome of this research is that biomonitoring based on traits could be reduced to one sampling event per year.

Incorporating traits into biomonitoring programmes offers the opportunity of developing a mechanistic framework relating the occurrence and distribution of traits in ecological communities to environmental stressors. Consequently, these traits could describe relevant processes involved in how environmental stressors affect communities (Rubach et al., 2011), thus providing insight into the source of impairment and informing management decisions.

6.5 Additional future directions

Evidence from my study (chapters 2 and 4) and others (e.g. Bhowmik and Schäfer, 2015, Logez et al., 2013) point to the fact that both trait and taxonomic composition of stream macroinvertebrates vary biogeographically. However, the effects of biogeography on taxonomic structure are typically stronger than traits. While some studies suggest that traits are able to overcome biogeographic effects (e.g. Statzner et al., 2004), however, very few studies using the same set of methodological and analytical approaches, have examined variation in trait and taxonomic composition across spatial scales. Therefore, further studies are needed to examine biogeographic congruence between taxonomic and trait patterns, and this could be expanded to other organism groups (e.g. macrophytes, macroinvertebrates and fish) and environmental settings.

Flow and salinity are globally held to be major drivers of aquatic communities, especially in dry environments (Williams, 2002). My study (Chapter 3) suggests that studies on effects of flow and salinity on macroinvertebrate traits were accumulating at a slow rate, with geographic coverage of studies strongly biased towards North America and Western Europe. The fewer number of studies and the geographic bias in research hinder efforts to

generalise about how salinity and flow affect macroinvertebrate traits. Given the projections of increased dry periods in many of the world's major river basins owing to climate change (Prudhomme et al., 2014), the global demand for water is predicted to increase (Van Huijgevoort et al., 2014). As a result, more research is needed urgently on flow-trait and salinity-trait relationships in under-represented regions (e.g. Asia and Africa) to establish baseline information that may help improve the diagnostic ability of bioassessment based on traits (Culp et al., 2011, Van den Brink et al., 2011).

While some databases already exist (e.g. Poff et al., 2006, Usseglio-Polatera et al., 2000b), there is still a paucity of trait data for many taxonomic groups and many types of traits, and this hinders the utilisation of traits. There is therefore the need for the establishment of effective and accessible trait databases (Baird et al., 2011). A well-documented, clearly defined, regularly updated source of trait information (Baird et al., 2011) resolved at genus or species level (Serra et al., 2016) and standardized in terms of trait classifications and nomenclature (Schmera et al., 2015) is needed. Further, there is the need for more definitive recommendations on preferred methodological approaches (Monaghan and Soares, 2014, Schmera et al., 2014) on how to relate traits to environmental conditions.

6.6 Conclusions

This thesis compared and contrasted taxonomic and trait based approaches in evaluating conditions in stream ecosystems, with a focus on the effects of flow and salinity. Flow and salinity were the most influential physical variables affecting macroinvertebrate taxonomic and trait composition in South Australia. The interactive effects of these stressors were also important, explaining some inconsistencies among studies in the trait literature. Flow and salinity variables explained more of the variation in trait-space than taxonomic, which is consistent with theory. However, in contrast to theory, there were still strong site-specific effects, which suggested that biogeographic effects remain, even for trait composition. Further, trait composition was more similar between seasons than taxonomic composition because there was more turnover in taxa than trait structure. This suggests that some traits are equally advantageous in both the dry and wet seasons. Thus, a management outcome of this research is that biomonitoring could be reduced to one sampling event per year if focussing on traits for environmental management guidelines is sufficient to address the monitoring goal. Additionally, differences in analytical and methodological approaches used to relate traits to environmental conditions pose challenges in trait-based analyses, and need to be

addressed in future studies. Finally, to foster the operative use of species traits for stream bioassessment, there is the need for comprehensive regional or national trait databases resolved at species level, and standardized in terms of trait classification and nomenclature.

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Supplementary material

Chapter 2

S1 Table. List of environmental, geographic and land use predictor variables

S2 Table. Spearman's correlations coefficients (ρ) between environmental, geographic and land-use predictor variables for the 13 sites. Abbreviations for predictor variables are listed in S1 Table. Bold numbers indicate $\rho > 0.90$ between variables for which reason one variable for chosen as a surrogate for the other variable.

S3 Table. List of 13 sites surveyed in this study.

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Table S1. Keywords used in my search for literature on trait responses to effects of flow and salinity

Table S2. Summary of flow-trait and salinity-trait studies used in our review. Table shows the range of salinity values covered for individual studies. Abbreviation in scale of study column represent; L: longitudinal scale; MC: multiple catchment; and C: catchment scale

Chapter 4

Table S1a. List of sites surveyed with their respective ionic proportions. Abbreviations and symbols represent: R (River); C (Creek); Agric (percent agriculture); Sym (symbol); the major cation and anion are sodium and chloride ions respectively.

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Table S2. Description of 26 traits in 75 modalities applied to 811 genera/ species in 185 families of South Australia freshwater macroinvertebrates categorized into four trait groups (bolded). The letter in each "Code" refers to the trait and the suffixed number refers to the trait state.

Table S3. Spearman's correlations coefficients (ρ) between flow variables and salinity for the 13 sites. Abbreviations for predictor variables are listed in appendix 2. Bold face indicates ρ > |0.70| between variables for which reason flowPerm was chosen over med.CTF

Table S4. Results of the multivariate generalized linear mixed models (MGLMM) for the 13 sites based on the trait states identified by BVSTEP for autumn and spring. Bold numbers indicate significant *P*-values. *df* indicate degrees of freedom.

Fig S1. Map of South Australia, showing the distribution of major land-uses and the sampling sites. Circles represent study sites; catchment boundaries upstream of study sites denoted by black lines; coastline and state borders in grey lines; thin black lines represent boundaries to NRM regions.

Fig S2. Canonical Analysis of Principal Component (CAP) ordinations overlaid with clusters to show the relative sensitivities of (A) taxonomic, and C) trait composition to geographic variation across multiple catchments. Ovals around site symbols show how sites cluster, and represent percentage similarity among the different clusters of sites. Different symbols represent different sites used in the study and their meaning are listed in Table S1 (▲: Bremer River; ▼: Finniss River; ■: First Creek; ♦: Hill River; ●: Hindmarsh River; +: Kanyaka Creek; ×: Light River; *: Marne River; Δ: Myponga River; ∇: North Para River; □: Rocky River; ◊: Scott Creek; ○: Torrens River)

Chapter 5

Table S1. Description of 26 traits in 75 modalities applied to 811 genera/ species in 185 families of South Australia freshwater macroinvertebrates categorized into four trait groups (bolded). The letter in each "Code" refers to the trait and the suffixed number refers to the trait state.

Table S2a. List of sites surveyed with their respective ionic proportions. Abbreviations and symbols denote: R (River); Ck (Creek); Agric (percent agriculture); Sym (symbol); the major cation and anion are sodium and chloride ions respectively.

Table S2b. List of sites surveyed with their respective mean ionic concentrations. Abbreviations and symbols denote: R (River); C (Creek).

Table S3. Salinity and flow metrics calculated for the sites included in the analysis. Min: minimum value; Max: maximum value; SD: standard deviation.

Table S4. Spearman's correlations coefficients (ρ) between flow variables and salinity for the 13 sites. Abbreviations for predictor variables are listed in Table 1 (Cond: salinity; Q75_30: high flow event > 75th percentile in the previous 30 days; flowPerm: flow permanence; DSLE: number of days since last low flow event; DSHE: number of days since last high flow event; and medQ: median flow). Bold face indicates ρ > |0.70| between variables for which reason flowPerm was chosen over med.CTF.

Table S5. Results of SIMPER (Similarity Percentages) analysis on trait composition for perennial and intermittent streams. Abbreviations denotes ("Av.Diss": average dissimilarity between season; "% Contr": percentage contribution of the respective trait modality to the total similarity between seasons for each stream type; "R": river; "Ck": creek). Values written against stream types denotes the total percentage dissimilarity between seasons for that particular stream type. Trait categories are arranged according to their decreasing contribution to the between-season dissimilarity. Abbreviations for trait categories are listed in Supporting Table S1.

Table S6. Table of variable importance weight in percentages (%) of perennial and intermittent sites for taxonomic and trait composition. Abbreviations for predictor variables are listed in Table S3 (Cond: salinity; Q75_30: high flow event > 75th percentile in the previous 30 days; flowPerm: flow permanence; DSLE: number of days since last low flow event; DSHE: number of days since last high flow event; and medQ: median flow)

Fig S1. Map of South Australia, showing the distribution of major land-uses and the sampling sites. Circles represent study sites; catchment boundaries upstream of study sites denoted by black lines; coastline and state borders in grey lines; thin black lines represent boundaries to NRM regions.

Supplementary material to Chapter 2

Table S1. List of environmental, geographic and land use predictor variables

Predictor variable	Constituent (%)	Symbol	Min	Max	Mean	sd
Environmental variable						
Fine sediment score (%)	Sand + Silt + Clay	FineSed	0	100	59.54	36.55
Algal cover score (%)		Algc	0	60	3.58	8.58
Detritus cover score (%)		Detc	0	100	30.55	24.12
pH		pH	5.63	8.73	7.70	0.58
Dissolved oxygen (mg/L)		DO	2.80	19.82	9.33	2.72
Conductivity (µS/cm)		Cond	139	23700	3709	4343
Local discharge (Runoff + drainage) (mm /day)		Runoff	4.7×10^{-6}	2.3×10^{-3}	0.0003	0.0004
Geographic variables						
Latitude (decimal degrees)		Lat	-35.95117	-32.096108		
Longitude (decimal degrees)		Long	136.708832	139.364501		
Catchment area above sample site (km^2)		CatchArea	9.18	826.37	221.72	220.98
Distance from source (km)		DFS	3.5	83.1	31.96	20.66
Land use variables based on ALUM categories						
(% area of upstream catchment)						
Conservation and minimal use (%)	Nature conservation + Other minimal uses	ConsvMin	0	98.23	21.78	30.09
Agriculture (%)	Cropping + Grazing modified pastures +	Agric	0.31	98.77	69.66	33.98

	Grazing natural vegetation + Intensive animal production + Intensive horticulture + Irrigated cropping + Irrigated modified pastures + Irrigated perennial horticulture + Irrigated plantation forestry + Irrigated seasonal horticulture + Perennial horticulture + Plantation forestry					
Urban (%)	Residential + Transport and Communication	Urban	0.64	32.60	6.90	8.50

S2 Table. Spearman's correlations coefficients (ρ) between environmental, geographic and land-use predictor variables for the 13 sites. Abbreviations for predictor variables are listed in S1 Table. Bold numbers indicate ρ > 0.90 between variables for which reason one variable was chosen as a surrogate for the other variable.

	Runoff	**Agric**	**ConsvMin**	**Urban**	**CatchArea**	**DFS**	**Lat**	**Long**	**Cond**	**DO**	**pH**	**FineSed**	**Algc**
Runoff													
Agric	-0.24												
ConsvMin	0.18	**-0.97**											
Urban	0.33	-0.44	0.23										
CatchArea	-0.21	0.36	-0.32	-0.25									
DFS	-0.25	0.45	-0.39	-0.39	**0.96**								
Lat	-0.07	0.57	-0.59	-0.29	0.08	0.11							
Long	-0.04	0.57	-0.67	-0.28	0.16	0.25	0.18						
Cond	-0.20	0.69	-0.68	-0.35	0.48	0.55	0.68	0.34					
DO	-0.06	0.13	-0.05	-0.13	0.01	0.02	0.30	0.02	0.20				
pH	-0.001	0.32	-0.38	-0.04	0.21	0.22	-0.40	0.32	0.49	0.25			
FineSed	0.05	-0.22	0.16	0.13	-0.09	0.00	-0.18	-0.31	-0.10	-0.22	-0.20		
Algc	-0.05	0.23	-0.15	-0.10	0.11	0.09	0.16	0.17	0.15	0.10	0.18	-0.26	
Detc	0.09	-0.24	0.11	0.19	-0.09	-0.03	-0.21	-0.08	-0.18	-0.21	-0.21	0.41	-0.21

S3 Table. List of 13 sites surveyed in this study

Site	Symbol	Latitude	Longitude
Bremer river	▲	-35.169208	139.021567
Finniss river	▲	-35.322908	138.66722
First creek	■	-34.971108	138.678886
Hill river	♦	-33.616457	138.62965
Hindmarsh river	●	-35.468682	138.585333
Kanyaka creek	+	-32.096108	138.291572
Light river	×	-34.358319	138.972061
Marne river	*	-34.653859	139.364501
Myponga river	Δ	-35.382156	138.475646
North Para river	∇	-34.46357	139.040276
Rocky river	□	-35.95117	136.708832
Scott creek	◊	-35.099448	138.67251
Torrens river	○	-34.795666	139.003485

Supplementary material to Chapter 3

Table S1. Keywords used in my search for literature on trait responses to effects of flow and salinity.

1	river* OR stream* OR freshwater* OR fresh-water* OR aquatic OR "aquatic ecosystem*" OR headwater* OR waterway*
2	Salinity* OR conductivity* OR salt*
3	macroinvertebrate* OR invertebrate* OR "aquatic invertebrate*" OR "aquatic macroinvertebrate*"
4	flow* OR hydrology* OR "flow intermittent*" OR "flow regime*"
5	"temporary stream*" OR "intermittent stream*" OR ephemeral* OR "permanent stream*" OR "intermittent river*" OR "permanent river*" OR "perennial stream*" OR "perennial river*"
6	trait* OR "biological trait*" OR "macroinvertebrate trait*" OR "invertebrate trait*" OR "life history trait*" OR "life-history trait*" OR "life history strategies*" OR "life-history strategies*"
COMBINED	(6 AND 5 AND 4 AND 3 AND 1); (6 AND 5 AND 3 AND 2 AND 1); (6 AND 5 AND 4 AND 3 AND 2 AND 1)

Table S2. Summary of flow-trait and salinity-trait studies used in our review. Table shows the range of salinity values covered for individual studies. Abbreviation in scale of study column represent; L: longitudinal scale; MC: multiple catchment; and C: catchment scale

Reference	Scale of study	Continent
Flow (N =11)		
Arscott et al. (2010)	L	Oceania
Brooks and Haeusler (2016)	L	Oceania
Cid et al. (2016)	MC	Europe
Chessman (2015)	MC	Oceania
Brooks et al. (2011)	MC	Oceania
Walters (2011)	L	North America
Bêche and Resh (2007)	L	North America
Bêche et al. (2006)	C	North America
Gallardo et al. (2009)	C	Europe
Garcia-Roger et al. (2013)	MC	Europe
Bonada et al. (2007)	MC	Europe
Salinity (N = 7)		
Gallardo et al. (2009) (4,000 µS/cm)	C	Europe
Szöcs et al. (2014) (High: 4,000-8,000 µS/cm; Low: > 1,500 µS/cm)	L	Europe
Piscart et al. (2006) (4,860 µS/cm)	L	Europe
Díaz et al. (2008) (9,000-50,000 µS/cm)	C	Europe
Kefford et al. (2012) (35,600 µS/cm)	MC	Oceania
Vidal-Abarca et al. (2013) (6,422 µS/cm - 130,084 µS/cm)	L	Europe
Schäfer et al. (2011) (12,500 µS/cm)	MC	Oceania

Supplementary material to Chapter 4

Table S1a. List of sites surveyed with their respective ionic proportions. Abbreviations and symbols represent: R (River); C (Creek); Agric (percent agriculture); Sym (symbol); the major cation and anion are sodium and chloride ions respectively.

Site	Sym	Latitude	Longitude	Agric (%)	Proportion of cations				Proportion of anions		
					Na^+	Ca^{2+}	Mg^{2+}	K^+	Cl^-	CO_3^{2-}	SO_4^{2-}
Bremer R.	▲	-35.169208	139.021567	81	0.731	0.124	0.111	0.034	0.999	0.00	1.0×10^{-5}
Finniss R.	▼	-35.322908	138.667220	76	0.681	0.153	0.149	0.017	0.971	0.029	0.00
First C.	■	-34.971108	138.678886	5	0.722	0.095	0.133	0.050	0.981	0.018	7.395×10^{-5}
Hill R.	♦	-33.616457	138.629650	96	0.761	0.074	0.155	0.100	0.983	0.017	0.00
Hindmarsh R.	●	-35.468682	138.585333	80	0.751	0.125	0.096	0.027	0.978	0.021	2.029×10^{-4}
Kanyaka C.	+	-32.096108	138.291572	99	0.750	0.105	0.133	0.012	0.993	0.007	0.00
Light R.	×	-34.358319	138.972061	91	0.758	0.086	0.136	0.020	0.990	0.010	0.00
Marne R.	*	-34.653859	139.364501	92	0.716	0.129	0.138	0.017	0.999	0	3.868×10^{-6}
Myponga R.	Δ	-35.382156	138.475646	85	0.673	0.180	0.126	0.021	0.980	0.020	0
North Para R.	∇	-34.463570	139.040276	91	0.692	0.140	0.142	0.026	0.994	0.006	0.00
Rocky R.	□	-35.951170	136.708832	0	0.795	0.039	0.142	0.023	0.995	0.005	0.00
Scott C.	◊	-35.099448	138.672510	20	0.550	0.203	0.222	0.025	0.969	0.031	0.00
Torrens R.	○	-34.795666	139.003485	86	0.718	0.129	0.127	0.026	0.949	0.051	0.00

Table S1b. List of sites surveyed with their respective mean ionic concentrations. Abbreviations and symbols represent: R (River); C (Creek); S.E (standard error)

Site	Cations (mg/L)				Anions (mg/L)		
	Na^{+}	Ca^{2+}	Mg^{2+}	K^{+}	Cl^{-}	CO_3^{2-}	SO_4^{2-}
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Bremer R.	542.8 ± 50.2	92.1 ± 6.5	82.5 ± 8.0	25.4 ± 2.3	933.8 ± 86.9	0.0 ± 0.0	0.009 ± 0.002
Finniss R.	176.3 ± 18.4	39.7 ± 6.0	38.4 ± 5.3	4.4 ± 0.4	358.0 ± 43.4	10.7 ± 10.7	0.006 ± 0.002
First C.	48.1 ± 2.6	6.3 ± 0.3	8.9 ± 0.6	3.3 ± 0.2	81.7 ± 5.3	1.5 ± 1.5	0.006 ± 0.004
Hill R.	1304.0 ± 192.8	126.8 ± 11.5	266.6 ± 38.4	16.9 ± 1.2	2432.2 ± 355.6	41.8 ± 37.5	0.005 ± 0.002
Hindmarsh R.	170.2 ± 18.4	28.3 ± 3.2	21.8 ± 2.2	6.1 ± 0.6	279.0 ± 31.1	6.1 ± 5.2	0.059 ± 0.026
Kanyaka C.	1363.6 ± 174.0	191.4 ± 14.8	241.2 ± 30.0	21.7 ± 2.4	2357.3 ± 319.0	17.2 ± 16.2	0.004 ± 0.001
Light R.	1370 ± 54.6	155.4 ± 4.9	246.9 ± 9.2	35.9 ± 2.0	2725.6 ± 119.3	28.5 ± 28.5	0.003 ± 0.000
Marne R.	719.1 ± 65.6	129.6 ± 11.4	138.2 ± 15.5	17.0 ± 1.7	1465.0 ± 148.6	0.0 ± 0.0	0.006 ± 0.002
Myponga R.	85 ± 6.6	22.7 ± 4.0	15.9 ± 1.7	2.7 ± 0.3	164.4 ± 15.1	3.4 ± 3.4	0.018 ± 0.007
North Para R.	530.0 ± 61.0	107.1 ± 9.0	109.1 ± 12.8	19.6 ± 1.8	982.4 ± 128.5	6.3 ± 4.2	0.076 ± 0.047
Rocky R.	80.9 ± 4.4	4.0 ± 1.1	14.5 ± 3.5	2.4 ± 0.3	142.3 ± 4.8	0.8 ± 0.8	0.003 ± 0.000
Scott C.	126.9 ± 10.0	46.9 ± 4.9	51.2 ± 5.4	5.7 ± 0.5	223.3 ± 21.8	7.2 ± 7.2	0.019 ± 0.002
Torrens R.	154.6 ± 32.3	27.7 ± 3.5	27.4 ± 4.9	5.5 ± 0.4	283.0 ± 63.8	15.2 ± 15.2	0.023 ± 0.006

Table S2. Description of 26 traits in 75 modalities applied to 811 genera/ species in 185 families of South Australia freshwater macroinvertebrates categorized into four trait groups (bolded). The letter in each "Code" refers to the trait and the suffixed number refers to the trait state.

Trait	Trait state (modality)	Code
Life history		
Voltinism	Semivoltine (< 1 generation/year)	Volt1
	Univoltine (1 generation/year)	Volt2
	Bi- or multivoltine (> 1 generation/year)	Volt3
Development	Fast seasonal	Devl1
	Slow seasonal	Devl2
	Non-seasonal	Devl3
Synchronization of emergence	Poorly synchronized (week)	Sync1
	Well synchronized (day)	Sync2
Adult life span	Very short (< 1 week)	Life1
	Short (<1 month)	Life2
	Long (>1 month)	Life3
Adult ability to exit	Absent (not including emergence)	Exit1
	Present	Exit2
Ability to survive desiccation	Absent	Desi1
Ability to survive desiccation	Present	Desi2
Physiological sensitivity to salinity (mS/cm)	Low < 7 mS/cm	Sal1
	Medium 7 – 20 mS/cm	Sal2
	med high 20 - 50 mS/cm	Sal3
	High >50 mS/cm	Sal4
Reproduction type	aquatic eggs	Rep1
	terrestrial eggs	Rep2
	ovoviviparity	Rep3
Time unitil reproduction (years)	< 0.5	Mat1
	0.5-1	Mat2
	>1	Mat3
Duration of life stages out of water	no terrestrial phase, obligate aquatic	Dur1
	has short terrestrial phase (weeks)	Dur2
	extended terrestrial life phase	Dur3
Metamorphosis	ametaboly	Meta1
	hemimetaboloy	Meta2
	holometaboly	Meta3
Mobility		
Adult dispersal	Low (<1 km flight before laying eggs)	Disp1
	High (>1 km flight before laying eggs)	Disp2
Adult flying strength	Weak (e.g., cannot fly into light breeze)	Flgt1
	Strong	Flgt2
Occurrence in drift	Rare (catastrophic only)	Drft1
	Common (typically observed)	Drft2
	Abundant (dominant in drift samples)	Drft3
Maximum crawling rate	Very low (< 10 cm/h)	Crwl1
	Low (< 100 cm/h)	Crwl2
	High (> 100 cm/h)	Crwl3
Swimming ability	None	Swim1
	Weak	Swim2
	Strong	Swim3
Morphology		
Attachment	None (free-ranging)	Atch1
	Some (sessile, sedentary)	Atch2
	Both	Atch3

Armouring	None (soft-bodied forms)	Armr1
	Poor (heavily sclerotized)	Armr2
	Good (e.g., some cased caddisflies)	Armr3
Shape	Streamlined (flat, fusiform)	Shpe1
	Not streamlined (cylindrical, round, or bluff)	Shpe2
Respiration	Tegument	Resp1
	Gills	Resp2
	Plastron, spiracle (aerial)	Resp3
Size at maturity	Small (< 9 mm)	Size1
	Medium (9–16 mm)	Size2
	Large (> 16 mm)	Size3
Ecology		
Rheophily	Depositional only	Rheo1
	Depositional and erosional	Rheo2
	Erosional	Rheo3
Thermal preference	Cold stenothermal or cool eurythermal	Ther1
	Cool/warm eurythermal	Ther2
	Warm eurythermal	Ther3
Habit	Burrow	Habi1
	Climb	Habi2
	Sprawl	Habi3
	Cling	Habi4
	Swim	Habi5
	Skate	Habi6
Feeding habit	Collector-gatherer	Trop1
	Collector-filterer	Trop2
	Herbivore (scraper, piercer, and shedder)	Trop3
	Predator (piercer and engulfer)	Trop4
	Shredder (detritivore)	Trop5

Table S3. Spearman's correlations coefficients (ρ) between flow variables and salinity for the 13 sites. Abbreviations for predictor variables are listed in appendix 2. Bold face indicates ρ > |0.70| between variables for which reason flowPerm was chosen over med.CTF

	Cond	**medQ**	**Q75_30**	**med.CTF**	**DSHE**	**DSLE**	**flowPerm**
Cond							
medQ	-0.36						
Q75_30	-0.28	0.53					
med.CTF	0.12	-0.27	-0.22				
DSHE	0.28	-0.26	-0.46	0.33			
DSLE	-0.26	0.37	0.49	-0.49	-0.47		
flowPerm	-0.40	0.47	0.33	**-0.75**	-0.40	0.63	

Table S4. Results of the multivariate generalized linear mixed models (MGLMM) for the 13 sites based on the trait states identified by BVSTEP for autumn and spring. Bold numbers indicate significant *P*-values. *df* indicate degrees of freedom.

	Autumn		
	df	***F***	***P***
Flgt2			
Site	12	3.42	**0.002**
Cond	1	2.52	**0.012**
DSLE	1	1.71	**0.046**
flowPerm	1	0.71	0.389
Cond × DSLE	1	9.72	0.100
Cond × flowPerm	1	0.67	0.413
Resp3			
Site	12	6.20	**0.001**
Cond	1	2.02	**0.015**
DSLE	1	0.21	**0.020**
flowPerm	1	2.06	0.153
Cond × DSLE	1	0.22	0.631
Cond × flowPerm	1	1.62	0.237
Resp2			
Site	12	3.64	**0.001**
Cond	1	1.46	**0.032**
DSLE	1	0.23	**0.021**
med.CTF	1	0.03	0.968
Cond × DSLE	1	2.12	0.151
Cond × flowPerm	1	0.13	0.701
Rheo3			
Site	12	1.64	0.105
Cond	1	0.50	0.469
DSLE	1	4.21	**0.031**

flowPerm	1	1.23	**0.028**
Cond × DSLE	1	4.19	0.411
Cond × flowPerm	1	0.29	0.599
Ther1			
Site	12	7.74	**0.001**
Cond	1	3.16	0.082
DSLE	1	0.30	**0.013**
flowPerm	1	1.10	**0.028**
Cond × DSLE	1	0.92	0.331
Cond × flowPerm	1	0.44	0.538
Habi1			
Site	12	8.30	**0.001**
Cond	1	2.32	0.122
DSLE	1	1.57	0.218
flowPerm	1	0.45	0.490
Cond × DSLE	1	5.87	**0.034**
Cond × flowPerm	1	0.54	0.466
Trop1			
Site	12	6.20	**0.001**
Cond	1	0.003	0.846
DSLE	1	0.60	0.472
flowPerm	1	0.40	0.546
Cond × DSLE	1	0.52	**0.045**
Cond × flowPerm	1	0.002	0.815
Rep3			
Site	12	16.39	**0.001**
Cond	1	0.64	**0.043**
DSLE	1	0.15	0.708
flowPerm	1	0.007	0.937
Cond × DSLE	1	1.18	0.296
Cond × flowPerm	1	0.15	0.696
Resp1			
Site	12	2.60	**0.005**
Cond	1	0.54	0.469
DSLE	1	0.06	0.803
flowPerm	1	1.06	0.326
Cond × DSLE	1	2.89	**0.014**
Cond × flowPerm	1	5.09	0.220
Spring			
Volt1			
Site	12	4.37	**0.001**
Cond	1	0.18	0.650
Q75_30	1	1.06	**0.021**
flowPerm	1	0.53	0.460
Cond × Q75_30	1	1.25	0.247
Cond × flowPerm	1	2.18	0.113
Volt3			
Site	12	8.48	**0.001**
Cond	1	0.84	**0.035**
Q75_30	1	4.95	0.028
flowPerm	1	0.18	0.683
Cond × Q75_30	1	5.69×10^{-5}	0.998
Cond × flowPerm	1	0.71	0.394

Crwl3			
Site	12	9.15	**0.001**
Cond	1	10.74	0.400
Q75_30	1	2.10	**0.003**
flowPerm	1	0.37	0.536
Cond × Q75_30	1	0.16	0.681
Cond × flowPerm	1	0.52	0.475
Habi1			
Site	12	7.75	**0.001**
Cond	1	11.03	0.001
Q75_30	1	1.23	0.268
med.CTF	1	1.53	0.232
Cond × Q75_30	1	1.49	**0.021**
Cond × flowPerm	1	0.73	0.401
Sal3			
Site	12	4.42	**0.001**
Cond	1	0.85	**0.037**
Q75_30	1	2.39	0.133
flowPerm	1	0.55	0.449
Cond × Q75_30	1	2.58	0.095
Cond × flowPerm	1	0.59	0.431
Mat3			
Site	12	7.99	**0.001**
Cond	1	1.87	0.188
Q75_30	1	0.09	0.762
flowPerm	1	2.54	**0.022**
Cond × Q75_30	1	0.12	0.705
Cond × flowPerm	1	1.69	0.200
Meta3			
Site	12	6.67	**0.001**
Cond	1	2.46	0.113
Q75_30	1	3.72	**0.050**
flowPerm	1	2.28	0.145
Cond × Q75_30	1	1.77	0.195
Cond × flowPerm	1	0.82	0.374
Shpe1			
Site	12	9.45	**0.001**
Cond	1	1.61	0.215
Q75_30	1	2.73	**0.048**
flowPerm	1	1.99	0.17
Cond × Q75_30	1	0.77	0.353
Cond × flowPerm	1	5.68×10^{-3}	0.935

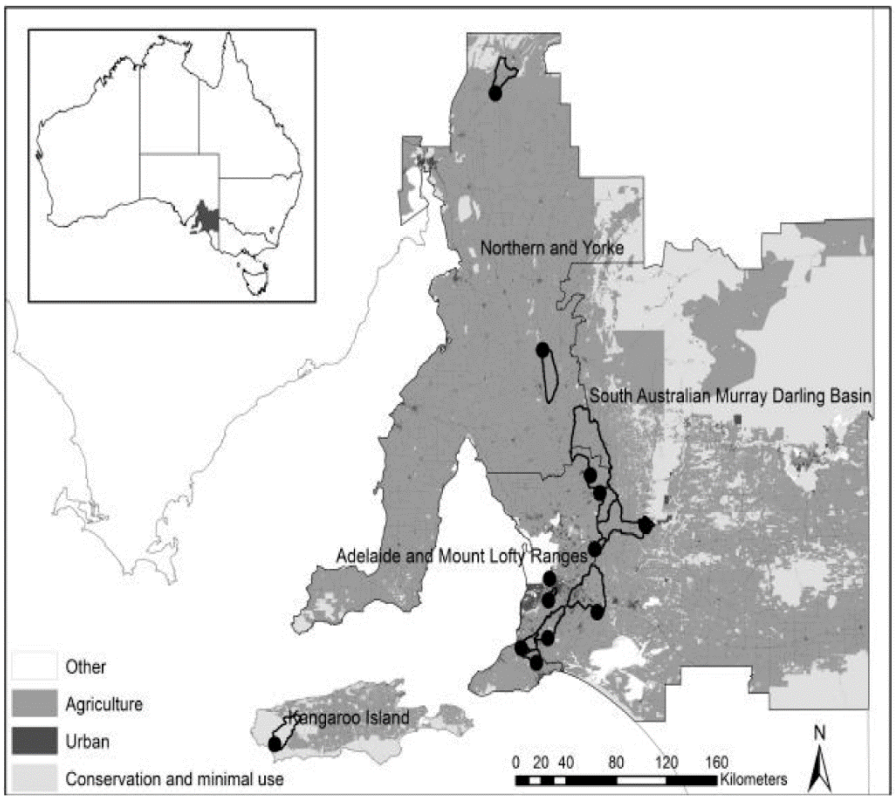

Fig S1. Map of South Australia, showing the distribution of major land-uses and the sampling sites. Circles represent study sites; catchment boundaries upstream of study sites denoted by black lines; coastline and state borders in grey lines; thin black lines represent boundaries to NRM regions.

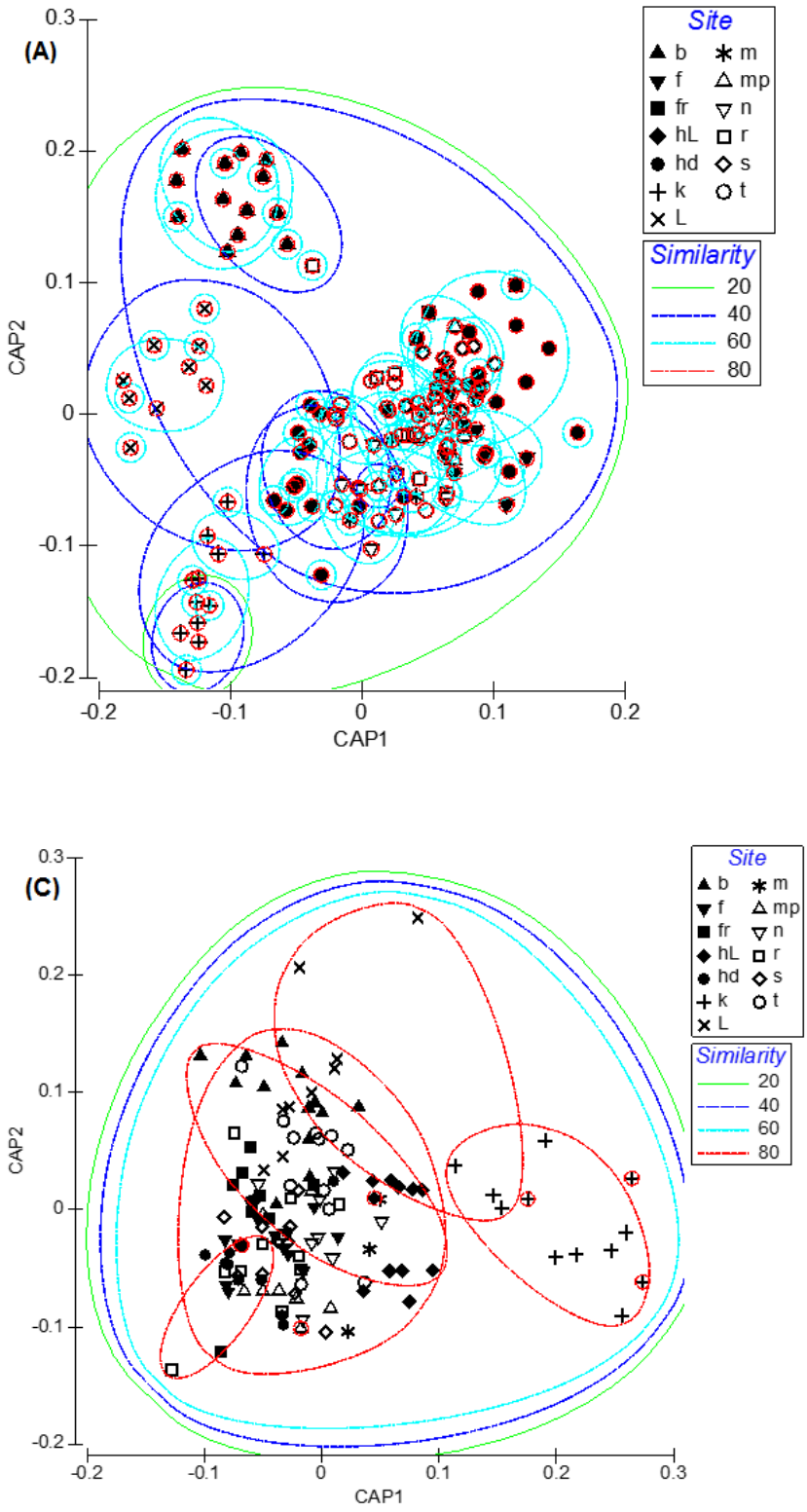

Fig S2. Canonical Analysis of Principal Component (CAP) ordinations overlaid with clusters to show the relative sensitivities of (A) taxonomic, and C) trait composition to geographic variation across multiple catchments. Ovals around site symbols show how sites cluster, and represent percentage similarity among the different clusters of sites. Different symbols represent different sites used in the study and their meaning are listed in Table S1 (▲: Bremer River; ▼: Finniss River; ■: First Creek; ♦: Hill River; ●: Hindmarsh River; +: Kanyaka Creek; ×: Light River; *: Marne River; Δ: Myponga River; ∇: North Para River; □: Rocky River; ◊: Scott Creek; ○: Torrens River)

Supplementary material to Chapter 5

Table S1. Description of 26 traits in 75 modalities applied to 811 genera/ species in 185 families of South Australia freshwater macroinvertebrates categorized into four trait groups (bolded). The letter in each "Code" refers to the trait and the suffixed number refers to the trait state.

Trait	Trait state (modality)	Code
Life history		
Voltinism	Semivoltine (< 1 generation/year)	Volt1
	Univoltine (1 generation/year)	Volt2
	Bi- or multivoltine (> 1 generation/year)	Volt3
Development	Fast seasonal	Devl1
	Slow seasonal	Devl2
	Non-seasonal	Devl3
Synchronization of emergence	Poorly synchronized (week)	Sync1
	Well synchronized (day)	Sync2
Adult life span	Very short (< 1 week)	Life1
	Short (<1 month)	Life2
	Long (>1 month)	Life3
Adult ability to exit	Absent (not including emergence)	Exit1
	Present	Exit2
Ability to survive desiccation	Absent	Desi1
Ability to survive desiccation	Present	Desi2
Physiological sensitivity to salinity (mS/cm)	Low < 7 mS/cm	Sal1
	Medium 7 – 20 mS/cm	Sal2
	med high 20 - 50 mS/cm	Sal3
	High >50 mS/cm	Sal4
Reproduction type	aquatic eggs	Rep1
	terrestrial eggs	Rep2
	ovoviviparity	Rep3
Time unitil reproduction (years)	< 0.5	Mat1
	0.5-1	Mat2
	>1	Mat3
Duration of life stages out of water	no terrestrial phase, obligate aquatic	Dur1
	has short terrestrial phase (weeks)	Dur2
	extended terrestrial life phase	Dur3
Metamorphosis	ametaboly	Meta1
	hemimetaboloy	Meta2
	holometaboly	Meta3
Mobility		
Adult dispersal	Low (<1 km flight before laying eggs)	Disp1
	High (>1 km flight before laying eggs)	Disp2
Adult flying strength	Weak (e.g., cannot fly into light breeze)	Flgt1
	Strong	Flgt2
Occurrence in drift	Rare (catastrophic only)	Drft1
	Common (typically observed)	Drft2
	Abundant (dominant in drift samples)	Drft3
Maximum crawling rate	Very low (< 10 cm/h)	Crwl1
	Low (< 100 cm/h)	Crwl2
	High (> 100 cm/h)	Crwl3
Swimming ability	None	Swim1

	Weak	Swim2
	Strong	Swim3
Morphology		
Attachment	None (free-ranging)	Atch1
	Some (sessile, sedentary)	Atch2
	Both	Atch3
Armouring	None (soft-bodied forms)	Armr1
	Poor (heavily sclerotized)	Armr2
	Good (e.g., some cased caddisflies)	Armr3
Shape	Streamlined (flat, fusiform)	Shpe1
	Not streamlined (cylindrical, round, or bluff)	Shpe2
Respiration	Tegument	Resp1
	Gills	Resp2
	Plastron, spiracle (aerial)	Resp3
Size at maturity	Small (< 9 mm)	Size1
	Medium (9–16 mm)	Size2
	Large (> 16 mm)	Size3
Ecology		
Rheophily	Depositional only	Rheo1
	Depositional and erosional	Rheo2
	Erosional	Rheo3
Thermal preference	Cold stenothermal or cool eurythermal	Ther1
	Cool/warm eurythermal	Ther2
	Warm eurythermal	Ther3
Habit	Burrow	Habi1
	Climb	Habi2
	Sprawl	Habi3
	Cling	Habi4
	Swim	Habi5
	Skate	Habi6
Feeding habit	Collector-gatherer	Trop1
	Collector-filterer	Trop2
	Herbivore (scraper, piercer, and shedder)	Trop3
	Predator (piercer and engulfer)	Trop4
	Shredder (detritivore)	Trop5

Table S2a. List of sites surveyed with their respective ionic proportions. Abbreviations and symbols denote: R (River); Ck (Creek); Agric (percent agriculture); Sym (symbol); the major cation and anion are sodium and chloride ions respectively.

Site	Sym	Latitude	Longitude	Agric (%)	Proportion of cations				Proportion of anions		
					Na^+	Ca^{2+}	Mg^{2+}	K^+	Cl^-	CO_3^{2-}	SO_4^{2-}
Finniss R.	▼	-35.322908	138.667220	76	0.681	0.153	0.149	0.017	0.971	0.029	0.00
Hill R.	♦	-33.616457	138.629650	96	0.761	0.074	0.155	0.100	0.983	0.017	0.00
Kanyaka Ck.	+	-32.096108	138.291572	99	0.750	0.105	0.133	0.012	0.993	0.007	0.00
Scott Ck.	◊	-35.099448	138.672510	20	0.550	0.203	0.222	0.025	0.969	0.031	0.00

Table S2b. List of sites surveyed with their respective mean ionic concentrations. Abbreviations and symbols denote: R (River); C (Creek).

Site	Cations (mg/L)				Anions (mg/L)		
	Na^+	Ca^{2+}	Mg^{2+}	K^+	Cl^-	CO_3^{2-}	SO_4^{2-}
	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Finniss R.	176.3 ± 18.4	39.7 ± 6.0	38.4 ± 5.3	4.4 ± 0.4	358.0 ± 43.4	10.7 ± 10.7	0.006 ± 0.002
Hill R.	1304.0 ± 192.8	126.8 ± 11.5	266.6 ± 38.4	16.9 ± 1.2	2432.2 ± 355.6	41.8 ± 37.5	0.005 ± 0.002
Kanyaka Ck.	1363.6 ± 174.0	191.4 ± 14.8	241.2 ± 30.0	21.7 ± 2.4	2357.3 ± 319.0	17.2 ± 16.2	0.004 ± 0.001
Scott Ck.	126.9 ± 10.0	46.9 ± 4.9	51.2 ± 5.4	5.7 ± 0.5	223.3 ± 21.8	7.2 ± 7.2	0.019 ± 0.002

Table S3. Salinity and flow metrics calculated for the sites included in the analysis. Min: minimum value; Max: maximum value; SD: standard deviation.

Parameter	Abbreviation	Description	Units	Min	Max	Mean	SD
		Finniss River (Perennial)					
Environmental variable							
salinity	Cond	Electrical conductivity, K_{25}	µS/cm	697	1920	1433.80	397.40
Hydrological regime							
Median flow	medQ	Median daily discharge on date of sampling	ML/day	1.9	15.84	6.50	3.50
Median cease-to-flow	med.CTF	Median daily cease-to-flow discharge over 365 days prior to sampling	ML/day	0.0	20	5.50	5.60
Annual average flow permanence	flowPerm	% time that flowing water was present over 365 days prior to sampling	%	80	100	0.90	0.05
Days since low flow event	DSLE	Number of days since a low flow event in 365 days prior to sampling	days	0.0	273	133.5	94.1
Days since high flow event	DSHE	Number of days since there was a high flow event in 365 days prior to sampling	days	0.0	241	57.6	83.0
High flow disturbance	Q75_30	Number of flow events exceeding 3× the 75th percentile daily discharge, 30 days prior to sampling	Count	0.74	81.41	19.44	20.64
		Scott Creek (Perennial)					
salinity	Cond	Electrical conductivity, K_{25}	µS/cm	484	1647	1165.57	355.84
Median flow	medQ	Median daily discharge on date of sampling	ML/ day	0.94	3.23	2.23	0.51
Median cease-to-flow	med.CTF	Median daily cease-to-flow discharge over 365 days	ML/ day	0	7	0.33	1.52
Annual average flow permanence	flowPerm	% time that flowing water was present over 365 days	%	70	100	0.99	0.07
Days since low flow event	DSLE	Number of days since there was a low flow event 365 days prior to sampling	days	32	304	167.71	90.62
Days since high flow event	DSHE	Number of days since there was a high flow event in 365 days prior to sampling	days	0	211	41.71	66.59

High flow disturbance	Q75_30	Number of flow events exceeding 3× the 75th percentile daily discharge, 30 days prior to sampling	Count	0.72	25.08	5.70	6.13
Hill River (Intermittent)							
salinity	Cond	Electrical conductivity, K_{25}	µS/cm	4280	13700	8009.19	2926.04
Median flow	medQ	Median daily discharge on date of sampling	ML/ day	0	1.45	0.10	0.33
Median cease-to-flow	med.CTF	Median daily cease-to-flow discharge over 365 days	ML/ day	8	355	111.5	78.78
Annual average flow permanence	flowPerm	% time that flowing water was present over 365 days	%	2	61	0.37	0.14
Days since low flow event	DSLE	Number of days since there was a low flow event 365 days prior to sampling	days	0	159	40.10	56.91
Days since high flow event	DSHE	Number of days since there was a high flow event in 365 days prior to sampling	days	0	301	132.38	111.30
High flow disturbance	Q75_30	Number of flow events exceeding 3× the 75th percentile daily discharge, 30 days prior to sampling	Count	0	12.26	2.79	3.98
Kanyaka Creek (Intermittent)							
salinity	Cond	Electrical conductivity, K_{25}	µS/cm	1470	22427	11467.83	6154.69
Median flow	medQ	Median daily discharge on date of sampling	ML/ day	0	0.36	0.11	0.12
Median cease-to-flow	med.CTF	Median daily cease-to-flow discharge over 365 days	ML/ day	9	205	62.32	60.96
Annual average flow permanence	flowPerm	% time that flowing water was present over 365 days	%	36	72	0.51	0.09
Days since low flow event	DSLE	Number of days since there was a low flow event 365 days prior to sampling	days	0	179	29.52	49.68
Days since high flow event	DSHE	Number of days since there was a high flow event in 365 days prior to sampling	days	0	261	91.61	83.58
High flow disturbance	Q75_30	Number of flow events exceeding 3× the 75th percentile daily discharge, 30 days prior to sampling	Count	0	0.66	0.28	0.20

Table S4. Spearman's correlations coefficients (ρ) between flow variables and salinity for the 13 sites. Abbreviations for predictor variables are listed in Table 1 (Cond: salinity; Q75_30: high flow event > 75th percentile in the previous 30 days; flowPerm: flow permanence; DSLE: number of days since last low flow event; DSHE: number of days since last high flow event; and medQ: median flow). Bold face indicates ρ > |0.70| between variables for which reason flowPerm was chosen over med.CTF.

	Cond	**medQ**	**Q75_30**	**med.CTF**	**DSHE**	**DSLE**	**flowPerm**
Cond							
medQ	-0.35						
Q75_30	-0.26	0.46					
med.CTF	0.07	-0.30	-0.04				
DSHE	0.20	-0.17	-0.39	0.20			
DSLE	-0.31	0.31	0.40	-0.25	-0.54		
flowPerm	-0.37	0.49	0.20	**-0.76**	-0.27	0.46	

Table S5. Results of SIMPER (Similarity Percentages) analysis on trait composition for perennial and intermittent streams. Abbreviations denotes ("Av.Diss": average dissimilarity between season; "% Contr": percentage contribution of the respective trait modality to the total similarity between seasons for each stream type; "R": river; "Ck": creek). Values written against stream types denotes the total percentage dissimilarity between seasons for that particular stream type. Trait categories are arranged according to their decreasing contribution to the between-season dissimilarity. Abbreviations for trait categories are listed in Supporting Table S1.

Finniss R. (Perennial: 14.34%)			**Scott Ck. (Perennial: 10.97%)**			**Hill R. (Intermittent: 15.76%)**			**Kanyaka Ck. (Intermittent: 17.92%)**		
Trait	**Av.Diss**	**% Contr.**	**Trait**	**Av.Diss**	**% Contr**	**Trait**	**Av.Diss**	**% Contr.**	**Trait**	**Av.Diss**	**% Contr.**
Habi6	0.34	2.35	Shpe1	0.21	1.95	Habi6	0.37	2.37	Habi6	0.40	2.24
Resp2	0.33	2.33	Mat3	0.18	1.67	Swim3	0.37	2.35	Mat2	0.39	2.16
Size3	0.33	2.32	Rheo3	0.18	1.67	Resp3	0.36	2.28	Life1	0.39	2.15
Armr1	0.33	2.31	Exit2	0.18	1.66	Rheo1	0.34	2.19	Habi4	0.38	2.13
Life3	0.33	2.30	Shpe2	0.18	1.63	Sal4	0.30	1.91	Devl1	0.38	2.13
Rheo1	0.33	2.28	Ther1	0.18	1.61	Crwl3	0.30	1.88	Desi1	0.38	2.11
Swim3	0.33	2.27	Volt2	0.18	1.60	Devl1	0.30	1.87	Dur2	0.36	2.02
Volt3	0.32	2.26	Sync1	0.17	1.58	Trop4	0.29	1.85	Resp2	0.36	2.01
Sync2	0.32	2.24	Drft3	0.17	1.57	Size2	0.29	1.83	Crwl2	0.36	2.01
Drft1	0.31	2.18	Ther2	0.17	1.57	Trop2	0.29	1.82	Drft1	0.35	1.98
Devl1	0.31	2.18	Rheo2	0.17	1.57	Crwl2	0.28	1.75	Swim3	0.35	1.95
Dur1	0.31	2.18	Rep1	0.17	1.57	Ther1	0.27	1.74	Size2	0.33	1.82
Sal2	0.31	2.17	Trop4	0.17	1.55	Mat3	0.27	1.73	Sal3	0.32	1.81
Desi2	0.31	2.16	Habi3	0.17	1.55	Life2	0.27	1.69	Exit1	0.32	1.79
Meta2	0.31	2.15	Flgt2	0.17	1.55	Armr1	0.26	1.65	Trop1	0.31	1.73
Exit2	0.31	2.14	Meta3	0.17	1.54	Mat2	0.26	1.63	Meta2	0.31	1.73
Crwl3	0.31	2.14	Dur2	0.17	1.53	Rheo3	0.26	1.63	Rheo1	0.30	1.69
Trop4	0.31	2.14	Swim1	0.17	1.52	Volt3	0.26	1.63	Armr2	0.30	1.67
Rep1	0.30	2.13	Disp2	0.17	1.52	Sync2	0.26	1.63	Volt2	0.30	1.65
Mat1	0.30	2.12	Flgt1	0.17	1.51	Life3	0.25	1.60	Disp1	0.29	1.62
Shpe2	0.30	2.12	Atch1	0.17	1.51	Trop5	0.25	1.56	Shpe2	0.29	1.61
Disp1	0.30	2.10	Resp1	0.17	1.51	Sal2	0.24	1.55	Atch1	0.29	1.61
Ther2	0.30	2.10	Size1	0.16	1.50	Habi3	0.24	1.55	Armr1	0.29	1.60
Atch1	0.30	2.10	Devl2	0.16	1.49	Desi2	0.24	1.53	Ther2	0.29	1.60
Flgt1	0.29	2.02	Exit1	0.16	1.48	Dur1	0.24	1.53	Flgt1	0.28	1.58
Flgt2	0.20	1.40	Disp1	0.16	1.48	Meta2	0.23	1.45	Volt3	0.28	1.56
Life2	0.20	1.38	Atch2	0.16	1.47	Exit2	0.22	1.43	Sal1	0.28	1.56
Mat3	0.18	1.29	Life1	0.16	1.46	Habi1	0.22	1.43	Swim2	0.28	1.55
Trop5	0.18	1.24	Swim2	0.16	1.45	Drft1	0.22	1.42	Rep2	0.28	1.55

Drft3	0.18	1.24	Habi6	0.16	1.45	Atch2	0.22	1.40	Sync1	0.27	1.51
Habi1	0.18	1.22	Desi2	0.16	1.44	Disp2	0.22	1.39	Sync2	0.27	1.49
Ther1	0.17	1.22	Devl1	0.16	1.44	Flgt2	0.22	1.39	Rep1	0.27	1.49
Devl2	0.17	1.21	Habi1	0.16	1.43	Rep1	0.22	1.39	Rheo2	0.27	1.48
Volt2	0.17	1.17	Meta2	0.16	1.43	Rep3	0.22	1.38	Drft3	0.26	1.45
Atch2	0.17	1.15	Crwl2	0.16	1.43	Size3	0.22	1.37	Dur3	0.25	1.38
Sal1	0.16	1.13	Sal3	0.16	1.43	Disp1	0.22	1.37	Crwl3	0.24	1.32
Habi3	0.16	1.10	Crwl1	0.15	1.41	Sal3	0.21	1.36	Disp2	0.23	1.31
Resp1	0.15	1.08	Dur1	0.15	1.41	Trop3	0.21	1.31	Trop4	0.23	1.30
Disp2	0.15	1.04	Sal1	0.15	1.40	Devl2	0.20	1.29	Resp3	0.23	1.29
Trop1	0.15	1.03	Mat1	0.15	1.39	Flgt1	0.20	1.28	Trop2	0.23	1.26
Rheo2	0.14	1.01	Resp2	0.15	1.39	Volt1	0.20	1.28	Exit2	0.22	1.25
Armr3	0.14	1.01	Life2	0.15	1.38	Atch1	0.20	1.26	Flgt2	0.22	1.25
Trop3	0.14	1.00	Drft1	0.15	1.38	Trop1	0.20	1.26	Life3	0.22	1.23
Swim1	0.14	1.00	Sal2	0.15	1.36	Habi2	0.20	1.25	Size1	0.22	1.21
Crwl2	0.14	0.99	Devl3	0.15	1.36	Ther2	0.20	1.25	Desi2	0.21	1.16
Sync1	0.14	0.96	Trop1	0.15	1.36	Mat1	0.19	1.23	Mat1	0.21	1.16
Trop2	0.13	0.94	Armr1	0.15	1.35	Shpe2	0.19	1.21	Rheo3	0.20	1.13
Swim2	0.13	0.91	Rheo1	0.15	1.34	Resp1	0.19	1.19	Ther1	0.20	1.12
Devl3	0.13	0.90	Crwl3	0.15	1.33	Sal1	0.18	1.17	Trop5	0.20	1.12
Crwl1	0.13	0.89	Desi1	0.14	1.32	Crwl1	0.18	1.16	Mat3	0.20	1.10
Ther3	0.13	0.89	Armr2	0.14	1.32	Habi5	0.18	1.16	Sal4	0.20	1.09
Resp3	0.13	0.89	Size2	0.14	1.31	Armr3	0.18	1.16	Rep3	0.19	1.06
Life1	0.13	0.88	Sync2	0.14	1.29	Dur2	0.18	1.12	Habi5	0.18	1.02
Dur2	0.13	0.88	Armr3	0.14	1.29	Drft3	0.17	1.10	Size3	0.18	1.01
Sal4	0.13	0.88	Trop5	0.14	1.27	Habi4	0.17	1.09	Life2	0.18	0.98
Meta3	0.13	0.88	Habi4	0.14	1.26	Swim2	0.17	1.08	Shpe1	0.18	0.98
Size2	0.13	0.87	Life3	0.14	1.24	Resp2	0.17	1.06	Drft2	0.18	0.98
Habi4	0.12	0.87	Resp3	0.13	1.22	Life1	0.17	1.06	Atch2	0.17	0.97
Desi1	0.12	0.87	Volt3	0.13	1.22	Devl3	0.17	1.05	Habi3	0.17	0.97
			Size3	0.13	1.21	Drft2	0.17	1.05	Dur1	0.17	0.95
			Drft2	0.13	1.20	Swim1	0.16	1.01	Devl3	0.17	0.93
			Trop2	0.13	1.20	Meta3	0.16	1.01			
			Trop3	0.13	1.16						

Table S6. Table of variable importance weight in percentages (%) of perennial and intermittent sites for taxonomic and trait composition. Abbreviations for predictor variables are listed in Table S3 (Cond: salinity; Q75_30: high flow event > 75th percentile in the previous 30 days; flowPerm: flow permanence; DSLE: number of days since last low flow event; DSHE: number of days since last high flow event; and medQ: median flow)

	Cond	**flowPerm**	**medQ**	**Q75_30**	**DSLE**	**DSHE**
Taxonomic composition						
Finniss River (Perennial)	27.75	26.42	26.87	24.20	23.93	25.13
Scott Creek (Perennial)	27.51	34.24	24.30	24.66	30.69	25.39
Hill River (Intermittent)	20.59	28.56	21.07	31.42	32.10	21.66
Kanyaka Creek (Intermittent)	34.86	24.91	19.12	32.81	29.08	18.81
Trait composition						
Finniss River (Perennial)	6.15	14.84	19.09	93.60	14.46	30.56
Scott Creek (Perennial)	34.82	36.36	14.71	29.68	32.03	16.28
Hill River (Intermittent)	30.01	19.83	25.64	47.65	33.54	14.13
Kanyaka Creek (Intermittent)	41.58	22.54	35.68	16.49	23.54	18.48

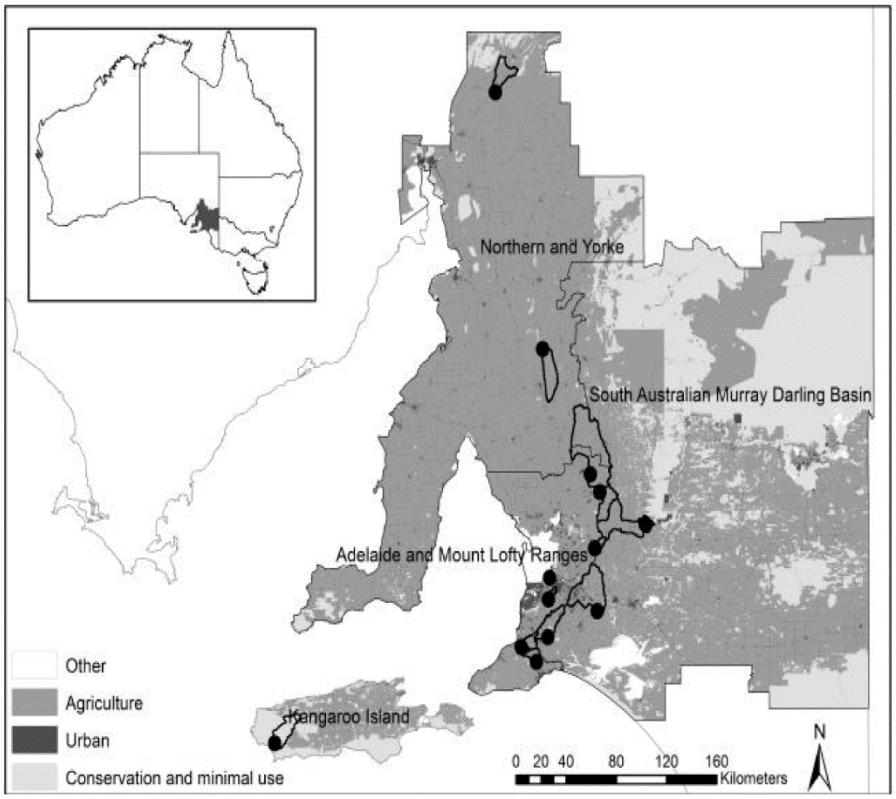

Fig S1. Map of South Australia, showing the distribution of major land-uses and the sampling sites. Circles represent study sites; catchment boundaries upstream of study sites denoted by black lines; coastline and state borders in grey lines; thin black lines represent boundaries to NRM regions.